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Volume 16 NOVEMBER-DECEMBER, 1951 Number 6

Food Research

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—NICOLAS APPERT.

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PRODUCT	Thiamine (B_1)		Riboflavin (B_2)		Niacin		Iron	
	MIN.	MAX.	MIN.	MAX.	MIN.	MAX.	MIN.	MAX.
Enriched BREAD, or other baked products	1.1	1.8	0.7	1.6	10.0	15.0	8.0	12.5
Enriched FLOUR[1]	2.0	2.5	1.2	1.5	16.0	20.0	13.0	16.5
Enriched FARINA[2]	1.66	–	1.2	–	6.0	–	6.0	–
Enriched MACARONI products[3]	4.0	5.0	1.7	2.2	27.0	34.0	13.0	16.5
Enriched NOODLE products[3]	4.0	5.0	1.7	2.2	27.0	34.0	13.0	16.5
Enriched CORN MEALS	2.0	3.0	1.2	1.8	16.0	24.0	13.0	26.0
Enriched CORN GRITS[4]	2.0	3.0	1.2	1.8	16.0	24.0	13.0	26.0
Enriched MILLED WHITE RICE[5]	2.0	–	*		16.0	–	13.0	–

1. *In enriched self-rising flour, calcium is also required between limits of 500-1500 mg. per pound.* 2. *No maximum levels have been enforced.* 3. *Levels allow for 30-50% losses in kitchen procedure.* 4. *Levels must not fall below 85% of minimum figures after a specific rinsing test described in the Federal Standards of Identity.* 5. *Levels must not fall below 85% of levels shown after washing and rinsing.*

**Omitted in the U.S.A. and Puerto Rico but used in certain Far Eastern countries to a minimum level of 1.2 mg. per pound.*

The maximum and minimum levels shown above for enriched bread, enriched flour, enriched farina, enriched macaroni, spaghetti and noodle products, enriched corn meal and corn grits are in accordance with Federal Standards of Identity or State laws. Act No. 183 of the Government of Puerto Rico requires the use of enriched flour for all products made wholly or in part of flour. This includes crackers, pretzels, etc.

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BACILLUS AS SPOILAGE ORGANISMS IN CANNED FOODS

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(Manuscript received July 3, 1950)

In a previous communication *(1)* we reported on anaerobic bacteria isolated from spoiled canned foods with a putrid content. The material for this investigation originated from sterility tests carried out with more than 10,000 cans including different products packed in various parts of Norway.

The present report supplements the investigation on putrefactive anaerobes with a taxonomic study on acid-producing organisms isolated from the same canned material. According to established statistics on frequency of occurrence of unsterility of the different products examined, the acid-producing group of bacteria is more important than the putrefactive anaerobes. They may be divided into: 1, flat-sour-producing organisms, and 2, acid- and gas-producing organisms. Of these groups the flat-sour-producing organisms are by far the most important.

According to the literature, acid-producing bacteria in canned foods are usually classified among the thermophiles, of which three major groups have been established:

1. Flat-sour-producing organisms, of which *Bacillus stearothermophiles* is the type species *(6)*.

2. Anaerobes not producing H_2S, representing organisms responsible for the kind of spoilage that is known as hard swells. The type organism is *Clostridium thermosaccarolytium (9)*.

3. Anaerobes producing H_2S, characterized by the production of H_2S. The type species is *Clostridium nigrificans (12)*.

Most research work on thermophiles has been carried out in the U.S.A. where temperature relations are favorable for the development of these organisms.

The presence of facultative thermophiles, including flat-sour- as well as acid- and gas-producing organisms, was shown in Norwegian canned vegetables purposely underprocessed, by Aschehoug and Vesterhus *(2)*. Of 523 cans, including 10 different kinds of vegetables, 121 strains of spoilage organisms were studied, showing flat-sour-, acid-, and gas-producing bacteria to be of great significance as spoilage organisms. None of these types were obligate thermophiles. Auto-sterilization of the cans was frequently encountered. Attempts were made to identify the acid-producing organisms according to Bergey's Manual, 5th edition, *(4)*, but none of the strains corresponded in all characteristics with any of the flat-sour-producing organisms described. *Bacillus subtilis* was identified as the organism responsible for "soft swells."

As for the mesophilic spore-forming aerobes of the genus *Bacillus*, they also include acid-producing bacteria. These organisms are widely distributed in nature, and are facultative anaerobes. Their presence in canned foods is explained by the great heat resistance of their spores. Aschehoug and Jansen *(1)* in their studies on putrefactive anaerobes state the frequent

presence of bacteria belonging to the *Bacillus subtilis* group together with putrefactive anaerobes in canned foods. According to literature, these organisms have usually been considered unimportant as spoilage agents, owing to the slight changes produced in the canned products, but opinions among the various investigators differ. Their significance has been evident in connection with dairy products and cured meats. Baumgartner *(3)* gives a review on the presence of spore-forming aerobes in canned foods.

EXPERIMENTAL PROCEDURE

The origin of the spoilage organisms studied were unsterile cans including the following marine products: fishcakes, fishballs, corned fish, mackerel, cod roe, and dressed crab. The meat products examined included beef, meat cakes, meat pudding, whalecakes, and blood pudding.

Prior to the sterility test, the cans were incubated for 10 days at 37°C.(98.6°F.), as we, according to earlier experience, very seldom encounter obligate thermophiles in Norwegian canned foods. In addition, most of these products are consumed in Norway where they hardly will be exposed to storage temperatures above 50°C.(122°F.).

Most of the cans were normal after the incubation period, although a minor group was blown (hard swells). The sterility test was carried out according to the classical method. After thorough cleansing with alcohol and flaming, the cans were punctured with a flamed steel rod, and samples of the contents were withdrawn by the use of Pasteur pipettes and inoculated into different enrichment media, such as nutrient broth, milk, deep meat medium, etc. for subsequent culturing under aerobic as well as anaerobic conditions. The contents of the unsterile cans were in all cases acid, the products having a pronounced acid odor and taste. The products were not disintegrated or decomposed, and showed no other signs of spoilage than acidification. Cans which were unsterile owing to post-process infection on account of leakage were excluded in this investigation.

The isolation and purification of the spoilage bacteria usually offered no difficulties. The spoilage organisms were often present in pure culture, possibly because of their acid production, which renders the media unsuitable for development of other organisms. In some cases a mixture of acid-producing bacteria was found. The spoilage organisms developed both under aerobic and anaerobic conditions. The bacteria were isolated by plating subsequent to culturing in different liquid and solid media.

Morphological and Cultural Characteristics

Identification of aerobic mesophilic spore-forming bacteria is combined with many difficulties, chiefly owing to inadequate differential tests. The work by Smith, Gordon, and Clark *(11)* has removed some of the difficulties, and their publication has in the present study been of great value for identification of the bacteria isolated. In addition Bergey's Manual, 6th Edition *(5)*, has been used as well as the last publication by Gordon and Smith *(8)*. We have also made use of Gibson's *(7)* contribution to the taxonomy of the genus *Bacillus*.

From about 200 strains 48 were selected as typical representatives of acid-producing organisms and subjected to a taxonomic study. The media used for identification of the strains were chiefly those recommended by Gordon and Smith *(8)*. For description of species the following characteristics were established: temperatures for growth; growth on proteose peptone acid agar; growth on citrate agar; growth on potato; growth in nutrient broth; growth in 5% NaCl broth; production of acetylmethylcarbinol; hydrolysis of starch; reduction of nitrate to nitrite; hydrolysis of gelatin; hydrolysis of casein; pH of glucose broth; fermentation tests (for the fermentation tests the ammoniacal nitrogen medium recommended by Smith, Gordon, and Clark *(11)* was used. Fermentation tests were carried out with the following carbohydrates: glucose, fructose, galactose, lactose, sucrose, maltose, dextrin, arabinose, xylose, inulin, glycerol, mannitol, and salicin); indol; hydrogen sulphide; relation to oxygen; and toxicity [toxicity studies were carried out on experimental animals (guinea pigs, rats, mice) by feeding, and on guinea pigs by injection].[a]

[a] Toxicity was tried out only on the strains identified as *Bacillus coagulans*.

As the key to the species of the genus *Bacillus* is based on the morphology of the sporangium and spores, as well as size of spores, much stress was laid on the microscopical examination of the strains.

The 48 organisms studied were according to their morphology, physiology, and biochemical reactions placed into 6 groups.

Group 1. This group included 17 strains. The morphology of the sporangia showed some variation, being slightly to definitely swollen. The spores were oval, subterminal to terminal. The biochemical properties corresponded well with *Bacillus coagulans*. Most of the cultures hydrolyzed starch, a few being negative. Reduction of nitrate was variable.

As to the maximum temperature of growth, bacteria developed at 55°C.(131°F.) at the time they were isolated. After having been kept at about 0°C.(32°F.) for a couple of years, however, the cultures had lost their ability of growing at 55°C., and had their maximum temperature at about 37°C.(98.6°F.). Several attempts were made to see if this lost ability could be regained. The cultures were incubated at 37°C., and were then placed at 55°C. After one day at that temperature, they were transferred to new broth and reincubated at 55°C. to see if the bacteria had been acclimatized. Most of the bacteria regained their ability of growing at 55°C.

Toxicity was tried out on experimental animals. The tests gave in all cases a negative result.

Group 2. This group included 9 strains. The sporangia were definitely swollen; clavate. The other characteristics corresponded with those of *Bacillus circulans*. Two strains differed in not hydrolyzing starch, but were not identifiable with the starch-hydrolyzing species, *Bacillus laterosporus* or *Bacillus brevis*.

According to various investigators, the *Bacillus circulans* group are complexes exhibiting variations in several directions. As most of the characteristics of the present cultures corresponded with *Bacillus circulans*, all the strains were considered members of this species.

Group 3. This group included 8 strains which differed from those in the preceding group in not fermenting arabinose and xylose. The organisms were not identifiable with *Bacillus alvei*, as the power of producing acetylmethylcarbinol was lacking. According to the established characteristics, the cultures were placed as *Bacillus circulans-Bacillus alvei* intermediates.

Group 4. This group included 9 strains which possessed the characters of *Bacillus subtilis*, and were consequently identified as such.

Group 5. This group included 2 strains which could neither be identified with the *Bacillus subtilis* nor with *Bacillus pumilus* on account of missing correspondence in reactions concerning nitrate reduction and starch hydrolysis with these species. Both cultures were placed in the group *Bacillus subtilis-Bacillus pumilus* intermediates.

Group 6. This group included 3 strains, the characteristics of which corresponded with those of *Bacillus macerans* and were identified as belonging to this species.

DISCUSSION AND CONCLUSIONS

The great importance of acid-producing organisms as spoilage agents in canned foods is generally recognized. From the present study on spoilage organisms originating from sterility tests carried out with more than 10,000 cans of different products, it appears that acid-producing organisms are of greater significance as spoilage agents than putrefactive anaerobes.

Forty-eight strains of acid-producing organisms, representing a collection of typical spoilage bacteria including flat-sour organisms as well as acid- and gas-producing ones, were, according to morphological and cultural characteristics subjected to a taxonomic study.

Identification of aerobic spore-forming bacteria is considered associated with considerable difficulties, owing to variations in cultures in different stages, inadequate differential tests, etc. Much confusion has been eliminated by the work of Smith, Gordon, and Clark *(11)*, and Gordon and Smith *(8)*.

One of the most important diagnostic features on which the identification of this genus is based is the morphology of the sporangia. Our group No. 1 includes cultures identified with *Bacillus coagulans*. The sporangia were slightly to definitely swollen, but all the other biochemical characteristics were in accordance with those of *Bacillus coagulans*. Even if this species should include organisms with spores not definitely swollen, our strains were identified as belonging to this species. Gordon and Smith *(8)* in describing characteristics of *Bacillus coagulans*, also mention strains with sporangia definitely swollen as well as strains with sporangia having no enlargement.

Difficulties were experienced with certain characteristics which seemed unstable, changing upon storage. Thus organisms classified in group 5 included one strain that, originally positive with regard to reduction of nitrate, gave a negative reaction after being kept stored at about 0°C. (32°F.) for a few years. Likewise one of the cultures in the same group which originally hydrolyzed starch, lost this ability upon storage.

As for the temperature relations, Gordon and Smith *(8)* have shown that maximum temperature of growth may vary widely among the strains of the individual species, and cannot be relied upon as a characteristic for identification of species, thus having little value from a taxonomic standpoint. We have had the same experience from the present investigation, as some organisms originally growing at 55°C., later lost their ability of growing at that temperature. Only upon successive transfers did we succeed in inducing the cultures to grow at 55°C.

In group 2 are included organisms showing most of the characteristics for *Bacillus circulans*. However, we did not succeed in demonstrating the rotary motion within the colonies on dried agar plates, neither with our strains, nor with an authentic strain from The American Type Culture Collection.

From the present study it appears that the identification system for aerobic mesophilic spore-forming bacteria is incomplete and unsatisfactory. Considering the importance of this genus, further work for establishing reliable descriptions of the different species is desirable and should include a great number of strains of the different species.

The organisms investigated included the following flat-sour-producing bacteria: *Bacillus coagulans* (17 strains), *Bacillus circulans* (9 strains), and *Bacillus circulans-Bacillus alvei* intermediates (8 strains).

Acid- and gas-producing organisms: *Bacillus subtilis* (9 strains), *Bacillus subtilis-Bacillus pumilus* intermediates (2 strains), and *Bacillus macerans* (3 strains).

Of these organisms the numerically most important species of the flat-sour-producing bacteria was *Bacillus coagulans*, of the acid- and gas-producing bacteria *Bacillus subtilis*.

Bacillus coagulans has been frequently encountered in canned foods. Thus Gordon and Smith *(8)* in their taxonomic study of aerobic spore-forming bacteria report about 95% of the *Bacillus coagulans* strains secured from different laboratories to originate from canned foods.

Concerning the significance of *Bacillus subtilis*, this organism was demonstrated by Michael and Tanner *(10)* in creamstyle canned corn. Of 256

strains of organisms isolated from this product, 251 were quite similar to the members of the *Bacillus subtilis* group, and produced "soft swells." In canned vegetables Aschehoug and Vesterhus *(2)* demonstrated organisms of the *Bacillus subtilis* group as frequently occurring spoilage organisms, in some cases producing "soft swells." In the present study evidence is given supporting our opinion that *Bacillus subtilis* is an important spoilage agent in canned foods.

Concerning the acid-producing organisms as contaminants in canned foods, their significance may be explained by their wide-spread distribution in nature. They have been isolated from the raw products used in the canning industry, such as milk, potato starch, etc., used as ingredients in preparing comminuted meat and fish products which are particularly liable to flat-sour spoilage. Their frequent occurrence in underprocessed canned foods is explained by the heat resistance which for some species is very high. In earlier experiments Aschehoug and Vesterhus *(2)* studied the heat resistance of an acid-producing organism isolated from canned peas. The heat resistance was 16 minutes at 115°C.(239°F.) and 4 minutes at 120°C.(248°F.). In the present study the heat resistance of the spores of 8 cultures was examined. One acid- and gas-producing strain survived 85 minutes at 105°C.(221°F.), and one flat-sour strain 160 minutes at 105°C., the latter culture thus having a very great heat resistance which compares with that of *Cl. sporogenes*. Repeated attempts to establish thermal death time curves failed, owing to difficulties with spore-formation and changes in heat resistance during cultivation and upon storage.

SUMMARY

The investigation includes a taxonomic study of 48 strains of flat-sour-, acid-, and gas-producing organisms representing a typical collection of spoilage organisms isolated from a variety of canned foods.

The flat-sour-producing organisms were identified with *Bacillus coagulans, Bacillus circulans,* and *Bacillus circulans-Bacillus alvei* intermediates.

The acid- and gas-producing organisms were identified with *Bacillus subtilis, Bacillus subtilis-Bacillus pumilus* intermediates, and *Bacillus macerans.*

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A STUDY OF BLACKENING AND FLUORESCENCE IN POTATOES[a,b]

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(Manuscript received May 29, 1950)

The appearance of a black or blue-black discoloration in potatoes after they are cooked and exposed to the air is a fault common in tubers grown in certain regions during certain seasons. It has been possible to reduce the discoloration in potatoes having this tendency by boiling them in acidified aqueous solutions *(1, 2, 4, 6, 9, 11, 12, 14)*. The favorable effect of many acids could be reversed by a change in hydrogen ion concentration; however, this was not true for two acids, boric and benzoic *(1, 2)*.

Ross *(10)* stated that browning in dehydrated potatoes was accompanied by the development of an ultra-violet fluorescence of the extracts. Lewis and Doty *(7)* obtained a blue fluorescent precipitate from tuber extracts and suggested that the blue fluorescent material was a precursor of the substance causing some cooked potatoes to turn black.

This study was undertaken to investigate further the relation of fluorescence to darkening. The investigations included an examination of both darkening and non-darkening potatoes and of potato extracts as well as the effect of various reagents on both darkening and fluorescence.

EXPERIMENTAL PROCEDURE

Unless otherwise specified, the potatoes were grown in Wisconsin, some being obtained on the retail market and some grown on the Agricultural Experiment Station farms and stored at 38° to 42°F. until used. Since it was never possible to predict whether a given tuber would turn dark after it was cooked nor which areas of the darkening tuber would show the discoloration, careful sampling and cooking techniques were necessary. For some comparisons, the potatoes were cubed, mixed, and portions taken for the various treatments; for other comparisons, halves, quarters or eighths cut lengthwise from the same tubers were used for the treatments to be compared. All cooked potatoes were exposed to the air for one hour at room temperature to permit maximum development of color before they were observed for blackening.

Whenever fluorescence and degree of blackening were to be correlated, tubers were bisected radiallly from bud to stem ends thus giving matching halves. One half of each tuber was cooked and discoloration noted. The other half was used for an extract on which the determination of fluorescence equivalent was made. Clear extracts were prepared from a slurry made by slicing the potato, then blending for one minute in a Waring Blendor with acidified alcohol (2 ml. per gram of potato; 400 ml. of 95% alcohol to 25 ml. of concentrated hydrochloric acid). For certain experiments, the raw half was fitted against the cooked half and the areas on the raw half corresponding to the black areas were selected for extraction.

Fluorescent regions were observed in the ultra-violet irradiation from a 100-watt Mazda B_4 Mercury Arc, using a filter of black glass transmitting light waves ranging

[a] Published with approval of the Assistant Director of the Wisconsin Agricultural Experiment Station.

[b] Financed in part by a grant from the Wisconsin Alumni Research Foundation.

[c] Division of Home Economics, Colorado A. and M. College, Fort Collins, Colorado.

from 2400 to 4000 A and with a maximum at 2800 to 3000 A. In order to obtain a quantitative measure of fluorescence, the transmission was measured in a Coleman photofluorometer fitted with B_1 and PC_1 filters. Fluorescence equivalence was computed in terms of micrograms of quinine sulfate used as standard of reference.

Absorption spectra curves were drawn from data obtained with a Beckman DU Spectrophotometer. The precipitate used for one curve was prepared and purified by the procedure of Lewis and Doty *(7)*.

RESULTS AND DISCUSSION

The fluorescence observable under ultra-violet irradiation was in certain varieties greatest at the stem ends of potatoes, whether raw or cooked. Sometimes, however, the fluorescent material appeared to have diffused into adjacent tissue. Often the most fluorescence was observed in the area where blackening occurred in the halves of the tubers which had been cooked and exposed to the air. This was especially true of Cobblers. There were, however, differences among potato varieties. In Chippewa and Sebago potatoes, there were small but highly fluorescent areas around the eyes and under the skin which frequently showed no black pigmentation in the cooked matching halves. Moreover, the Russet Burbank variety tended to show little fluorescence even in the Wisconsin grown potatoes that blackened markedly.

TABLE 1

Fluorescence of extracts from darkening and non-darkening areas in matched halves of Wisconsin Cobbler potatoes

Tuber No.	Fluorescence equivalent,[d] γ/g. potato		
	Raw non-dark areas[e]	Raw dark areas[e]	Cooked dark areas[e]
1	5.57	9.86	5.57
2	6.81	12.00	9.00
3	.....[f]	12.21	9.86
4	6.43	8.57	8.57
5	6.81	12.00	9.00
Avg	6.39	10.93	8.40

[d] Computed as micrograms equivalent of quinine sulphate.
[e] Non-darkening and darkening portions in raw half of potato were cut from areas corresponding to non-darkening and darkening regions in the cooked half.
[f] Too little area for sampling.

A quantitative measurement was made of the fluorescence of extracts made from darkening and non-darkening areas of Cobblers. Less fluorescence was noted in those portions of the tubers having less darkening (Table 1). The fluorescence equivalents (in relation to quinine sulfate) of the cooked dark areas were lower than for the raw. Losses in the cooking water may have caused this difference since the fluorescent material of potatoes was very soluble.

The possibility of a relationship between fluorescence and darkening was studied further by correlating the fluorescence equivalents of extracts from raw tubers with the color of their cooked matched halves. The data are summarized as follows: White or creamy white tubers had a fluorescence level which ranged from 0.43 to 1.71; nine tubers, showing slight graying about the stem end ranged from 0.86 to 5.14 in fluorescence equivalents; ten tubers which were rated as having moderate darkening, 0.75

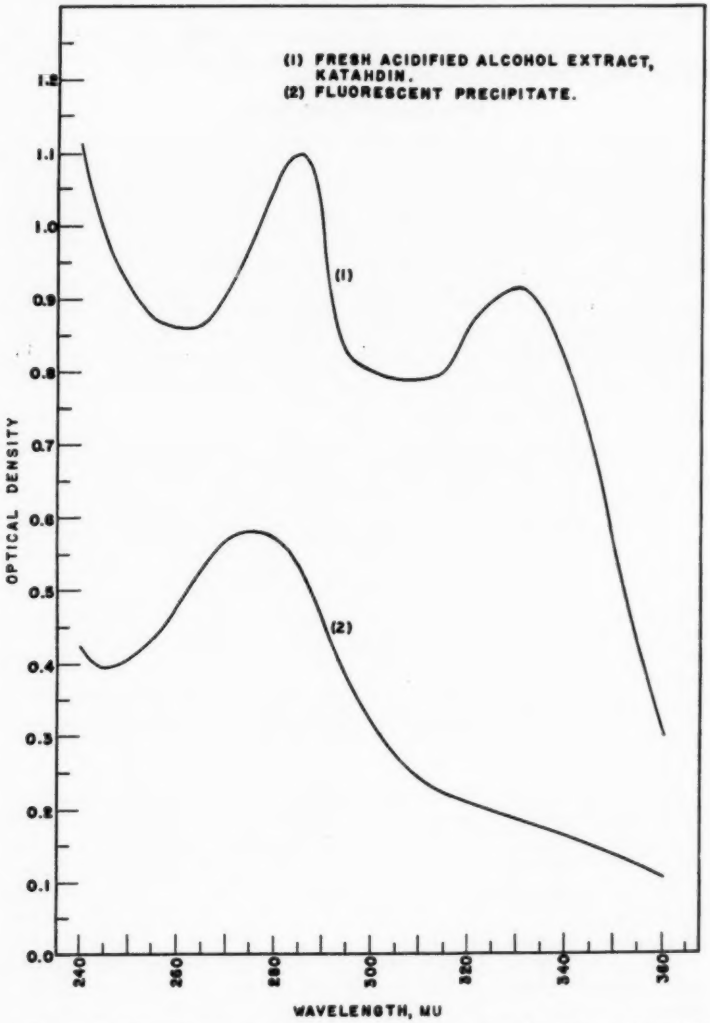

FIGURE 1. Absorption spectra for a fresh acidified alcohol extract from Katahdin and for the blue fluorescent precipitate obtained from Cobbler potatoes.

to 5.89; whereas, eleven tubers severely darkened, ranged from 1.07 to 10.50 in fluorescence equivalents. The potatoes which darkened most were Wisconsin-grown, and in general had higher fluorescence values, yet there were other tubers which showed no such relationship. For example, of 6 tubers which had a fluorescence equivalent of 1.71, one was white, one severely darkened, and four showed moderate darkening.

Another approach to the possible relation of darkening and fluorescence was through the comparison of absorption spectra. As a first step, a precipitate was prepared by the method of Lewis and Doty *(7)* from Wisconsin Cobblers which cooked dark. The absorption spectrum of this precipitate as given in Figure 1 is similar to that reported by Lewis and Doty *(7)*. Included also in Figure 1 is a curve which represents the absorption measurements of a fresh extract prepared from Wisconsin Katahdin potatoes that cooked dark. Both curves have maxima in the neighborhood of 280 mμ. but that of the fresh extract has another lesser peak at 330 mμ. Furthermore, our experiments showed that reaction of the extract of the Cobbler potatoes with bromine resulted in the loss of the maximum at 280 mμ. as Lewis and Doty *(7)* had reported for their precipitate. Potato extracts made with acidified alcohol were therefore used for all further work on absorption spectra.

The curves of Figure 2 demonstrate that the ultra-violet absorption spectra were similar for extracts from two lots of Russet Burbank potatoes, each having a peak at about 280 mμ. One lot was grown in Idaho and cooked white; the other lot was grown in Wisconsin and cooked dark. Whereas both the precipitate and extracts prepared from potatoes that blackened markedly reached a characteristic peak at 280 mμ. (Figure 1), these same maxima are present in the curves shown in Figure 2. Thus for one variety, absorption curves of extracts prepared from potatoes of a non-darkening lot, as well as those from a darkening lot, likewise reached maxima at about 280 mμ. In every potato extract for which an absorption curve was determined, the maximum at about 280 mμ. was present.

The third phase of this study between the relationship of fluorescence and darkening in potatoes was to observe the effect of added substances which may be related to either fluorescence or darkening. Haas, Stadtman, Stadtman, and Mackinney *(5)* reported that a wide variety of darkened foods gave extracts which showed a characteristic band at about 285 mμ. It has been demonstrated that the darkening (browning) of dried apricots is associated with the production of furfural *(8, 13, 15)*. Therefore freshly redistilled furfural was added to potato extracts and the absorption spectrum determined. The effect of furfural addition was to intensify the absorption and with the same peak occurring at about 280 mμ. Extract of potatoes treated with furfural became brown but in no case was a black color obtained.

Since a difference had been found in the effect on the reversibility of the dark color of cooked potatoes, treated with boric or benzoic acids in contrast to those treated with cream of tartar *(1, 2)*, the fluorescence of extracts from potatoes cooked in these was studied. Comparable samples of the stem-end portions of Cobbler potatoes that cooked dark were boiled

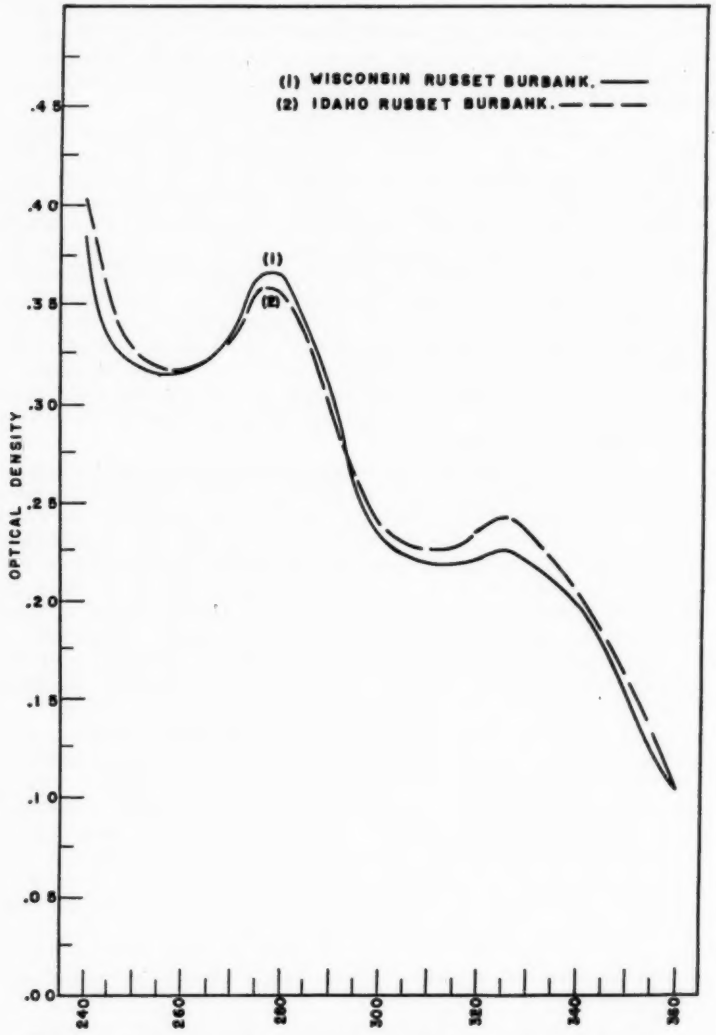

FIGURE 2. Absorption spectra for extracts obtained from two lots of potatoes of the same variety. Curve (1): Russet Burbank potatoes grown in Wisconsin turned dark after cooking. Curve (2): Russet Burbank potatoes grown in Idaho remained white after cooking.

in solutions of boric or benzoic acid, cream of tartar, or in distilled water. The fluorescence equivalents (relative to quinine sulfate) were 2.64 γ/g. of potato in distilled water but 3.43 γ/g. for all of the other samples. Thus boric and benzoic acids which inhibited darkening did not decrease fluorescence.

A fourth approach to this problem was an attempt to characterize the fluorescent material by partition and paper chromatographic methods. It was found that the greater portion of the fluorescent material could be extracted from a water solution by ethyl acetate or by ether. These findings were in accord with the characteristics noted by Haas et al. *(5)* and Wahab *(15)* when working with the fluorescent material of browned dried fruit.

The fluorescent materials from water or aqueous extracts of darkening potatoes were taken out by ether and then concentrated by vacuum distillation below 30°C. The concentrate was then subjected to the procedures used by Dent, Steppa, and Stewart *(3)* for the detection of amino acids in plant material by partition chromatography. It was found that the fluorescent material migrated in both solvents. After drying, the fluorescing portions of the paper were steamed over ammonium carbonate vapors for 30 minutes, the procedure that according to Tinkler *(14)* increased the blackening in cooked potatoes. The paper strips showed no evidence of blackening but slight browning developed. A negative test for amino acids was obtained by spraying with ninhydrin reagent. The blue fluorescent precipitate which had been prepared and for which an absorption spectrum had been determined was likewise studied for migration on the paper chromatograph and for color. The results were the same as for the potato extracts.

In this investigation, all attempts to produce or to intensify blackening in potatoes by treatment with amino acids or sugars or related compounds were unsuccessful. The fluorescence, however, was not determined. Browning developed after potatoes were treated with glycine or furfural but a gray or black pigmentation never formed.

SUMMARY

A precipitate prepared from potatoes that darkened after cooking was found to have a blue fluorescence and an absorption peak at 275 to 285 mμ. The absorption spectra for extracts from darkening and non-darkening Russet Burbank potatoes were almost identical. The characteristic peak at about 280 mμ. was destroyed by the addition of bromine, and increased by furfural. The extracts with added furfural turned brown in color but not black or gray. A lack of correlation was found between fluorescence and blackening when cream of tartar or boric or benzoic acids were added to inhibit darkening. The fluorescent spots on paper chromatographs, prepared from the fluorescent precipitate or from extracts of potatoes that darkened, turned slightly brown but not black or gray when steamed over boiling ammonium carbonate solution. The results of these experiments indicate no clear relationship between the fluorescence and the tendency of certain potatoes to turn dark after they are cooked.

Acknowledgment

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EFFECT OF HAND AND MACHINE MASSAGE ON THE TENDERNESS OF POULTRY MUSCLES AGED FOR SHORT-TIME PERIODS[a]

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The effect of many of the large-scale production-line methods introduced into the poultry packing industry is not known. Defeathering machines may exert a massage-like action upon the poultry carcass which may in turn influence the subsequent tenderness of the meat. The basis for such assumption lies in a study reported by Callow *(2)*. Callow found that when the excised psoas muscle of pork was massaged for 30 seconds the electrical resistance of the tissues was reduced by half, a change similar to that accompanying aging. It would appear that massage brings about internal changes in the muscles similar to those produced by aging. Whether or not it also produces the tenderizing effect of aging merits investigation.

Previous work in this laboratory *(3, 4, 6)* has shown that poultry muscle tenderizes rapidly during aging. Hence, any tenderizing effect of the picking machines or hand massage should be noticeable on birds aged for short periods, the cooking of which is started within 30 to 60 minutes after slaughter. Longer aging periods may mask any tenderizing effect of the massage by the picking machines.

EXPERIMENTAL

The 99 roasters (one bird was discarded because of pathological condition) used in the experiment were raised at the College poultry farm. They consisted of 47 Barred Plymouth Rocks and 52 New Hampshires. The roasters were 7 to 9 months old at time of slaughter. To somewhat equalize size at the time of slaughter, the larger birds in the flock were used first. Otherwise the birds were selected and assigned to the different treatments arbitrarily, irrespective of breed.

Design of experiment. Seven groups of birds were held 30 minutes after slaughter before placing them in the oven, two groups were held 60 minutes. The assignment of treatments follows:

The seven groups, held for 30 minutes, were: A, control group (picked by hand), 20 birds; B, massaged by hand 4 minutes, 10 birds; C, massaged by hand 8 minutes, 10 birds; D, massaged (picked) by machine I, 15 seconds, 10 birds; E, massaged by machine I, 60 seconds, 10 birds; F, massaged by machine II, 15 seconds, 10 birds; and G, massaged by machine II, 60 seconds, 10 birds.

The two groups, held for 60 minutes, were: H, picked by hand, control, 9 birds; and I, massaged by hand, 4 minutes, 10 birds.

Slaughter. The birds were shackled by the feet and hung head downward. The arteries of the throat were cut through the mouth and the bird was bled for 1 minute. Immediately after making the incision, an electric stunning device was attached to the wattles and current was supplied for 5 seconds (10 seconds for the older and heavier birds). The application of the current produced a spasmodic jerk followed for a few seconds by convulsive quivering and a general relaxing of the body. In some cases, the bird struggled throughout the bleeding period, the scalding treatment and/or the initial

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picking, that is, for 3 or 4 minutes after slaughter. The bird was slack scalded for 1 minute in approximately 5 to 5.5 gallons of water, maintained at 54.4°C.(130°F.) and containing a wetting agent, sodium lauryl sulfonate. During the scalding period the bird was vigorously agitated in the water.

Picking. The control groups and those to be hand massaged were rehung in the shackles and hand picked by two operators. This required an average of 4.5 minutes.

The machine-picked birds were removed from the scalding water and placed in a picking machine. This required approximately 5 seconds. Machine I consisted of a steel frame which was surmounted by a horizontal shaft drum bearing flexible fingers. The operator held the bird by the feet with its body laid over the rubber fingers of the rotating drum. The body was rolled slightly from side to side so that all parts received equitable treatment. The bird was laid on its back half of the time and on its breast half of the time. The large wing and tail feathers were removed by hand.

Machine II bore two vertical shaft drums placed side by side so that the extended rubber fingers were slightly overlapping at the bottom, not quite touching at the top. The bird was held in this area by holding the feet with one hand, the neck with the other. It was held breast down for half time and back down for half time. The large wing and tail feathers were pulled before the carcass was placed between the rotating drums.

The 15-second period on either machine was sufficient to remove the larger feathers from the body. The additional time with the 60-second treatment was largely massage.

After removing from the machine, the birds were immediately rehung in the shackles and the smaller feathers remaining on the neck, legs, and wings were removed by hand. The total picking time averaged about 4 minutes.

Hand massage. After picking, the bird was placed on its back on a table, head toward the operator. The operator grasped the wings and rotated them, moving them simultaneously at the rate of about one revolution per second for a period of 2 minutes. A second operator assisted by holding the feet of the bird on the table. At the end of 2 minutes the position of the bird was reversed and the legs rotated alternately, bicycle fashion at the same rate for 2 minutes. For the 8-minute massage period, these two processes were repeated.

Cooking. After evisceration and washing the carcass was put breast down on a V-shaped chromium rack placed in a shallow pan. The oven temperature was 150°C. (about 300°F.). The bird was removed from the oven when the internal thigh temperature reached 90.5°C.(195°F.).

Loss in weight during cooking and the cooking time were also determined.

Palatability. Cross sections, about ⅜ inch in thickness, of the two breast muscles (the pectoralis major and pectoralis secundus) and of the composite thigh muscles were used for palatability ratings. The scoring panel consisted of four judges. Comparable sections from each bird were given a specific score. The factors scored were aroma, flavor, juiciness, and tenderness. Each factor was rated 0 to 10, with 10 high. As an aid in estimating tenderness, each scorer counted the number of chews to masticate the sample. For a definite number of chews the tenderness rating was lowered by one point. The breast muscles from the left side of the bird were used for shearing tests.

RESULTS

The dressed weight of the 99 carcasses varied from 1585 to 2194 g. (without giblets) and averaged 2055 g.

The average time for the thigh temperature of the 99 birds to reach 90.5°C.(195°F.) during cooking varied from 90 to 192 minutes and averaged 128 minutes.

Palatability

Aroma, flavor, and juiciness scores. There was very little variation in the aroma scores. The average flavor scores were slightly lower than those for aroma. Variation occurred in the flavor and juiciness scores from bird to bird, but the massage treatments did not produce significant differences in the aroma, flavor, or the juiciness scores.

Tenderness, scores. The pectoralis secundus, one of the most tender muscles in the bird, was scored the most tender; the thigh muscles were scored as least tender; and, the pectoralis major as intermediate (Table 1).

TABLE 1

Average tenderness scores and shear force of groups of roasters subjected to various massage treatments and scores of birds that struggled during slaughter versus those not struggling

Group No. and treatment	Tenderness scores			Shear force	
	Pectoralis major	Pectoralis secundus	Composite thigh	Pectoralis major	Pectoralis secundus
				lbs.	*lbs.*
Aged 30 minutes					
A. Control	4.0	7.7	2.4	42.9	16.2
B. Hand-massaged, 4 minutes	5.0	7.6	3.6	39.5	16.4
C. Hand-massaged, 8 minutes	5.4	8.1	3.7	40.2	13.6
D. Machine I, 15 seconds	4.8	6.5	3.2	49.6	17.9
E. Machine I, 60 seconds	5.4	7.2	3.4	47.0	16.1
F. Machine II, 15 seconds	5.3	7.8	4.1	35.6	12.0
G. Machine II, 60 seconds	5.4	7.6	3.4	39.1	16.7
Aged 60 minutes					
H. Control	6.0	8.4	4.7	34.7	13.6
I. Hand-massaged, 4 minutes	6.2	8.2	4.7	34.6	14.2
All birds					
19 struggling	6.0	8.3	4.6	34.1	13.3
80 not struggling	5.0	7.5	3.3	42.2	15.8

A study of the average scores of the birds of groups aged 30 minutes shows that the muscles from hand- and machine-massaged groups of birds were tenderer than those for the control group. The analyses of variance showed that the differences between the tenderness scores of the control and the massaged groups were significant at the 1% probability level for the pectoralis major and thigh muscles, but not for the pectoralis secundus. Two factors may have prevented the pectoralis secundus from responding to the massage treatments. It, like beef tenderloin, is always, even without aging, a tender muscle and hence may not respond to massage treatment. The secundus is a smaller muscle than the major and lies under the major, and may have been protected from the massage treatment. There was, however, wide variation in the tenderness of a given muscle from bird to bird. This variation in rate of tenderizing during aging has been encountered in three previous studies *(3, 4, 6)* in this laboratory. It has been found that, in general, breast muscles of poultry tenderize very rapidly (to 3 hours for broilers, 6 hours for roasters) and more slowly thereafter. The thigh muscles tenderize far more slowly than the breast muscles. Within a group of birds (broilers for example) the muscles of some birds tenderize more rapidly, those of other birds more slowly. The reliability of these findings was indicated by the histological changes in the muscle fibers which accompanied the changes in tenderness *(5)*.

Tenderness, shear force. Five shears were made on the pectoralis major and on the pectoralis secundus. No thigh muscles were sheared. There was close agreement between the tenderness scores and shear force values of the pectoralis secundus muscle, but not for the pectoralis major. The

regression coefficient for tenderness scores and shear force value for the pectoralis major was determined, r being —0.51, which was significant at the 1% probability level. This indicated that as the shear force in pounds increased the tenderness scores became lower.

The two groups (D and E) of birds picked on machine I required a higher average number of pounds for shearing than the control group. It is known that the force for shearing is affected by the size of the muscle, a thick muscle requiring more pounds to cut through it than an equally tender, thin muscle. One muscle may be long and thin, yet weigh the same as a shorter, thicker one. However, the weight of the muscle does give some indication of its size. The weight of the pectoralis major used for shear purposes varied from 29 to 107 and averaged 78 g. By chance distribution the average weight of the pectoralis major of birds in groups D and E was considerably heavier than the average of all other groups, with exception of group B. In addition no birds of group D and E struggled much at the time of slaughter. The weight of all the muscles used for shearing was statistically adjusted to a common weight to see if this would bring the shear force values in agreement with the tenderness scores for the pectoralis major. The differences between the shear force for the control and the treated groups were still nonsignificant, although the correlation between the weight of the muscle and its shear force in pounds was significant at the 1% level, r being 0.49.

Tenderness after aging for 30 or 60 minutes. The average tenderness scores of the pectoralis major, the pectoralis secundus, and the thigh muscles of the carcasses from the groups aged 60 minutes before cooking indicated these muscles were more tender than those from the corresponding groups (A and B) aged 30 minutes. These differences were highly significant. Hand massaging for 4 minutes of the carcasses which were aged 60 minutes did not increase the tenderness over that for the control group. Thus aging 60 minutes had more effect than the massage treatments upon tenderness of the muscles.

Tenderness as affected by struggle at the time of slaughter. Bate-Smith *(1)* states that "The process of acidification of the muscle commences sharply with the cessation of the blood supply to the muscles. . . ." "The precise pH of the muscle prior to cessation of the circulation depends on the recent history of muscular activity. If the muscles have been completely inert and well supplied with oxygen for a good while before death, metabolism will have been completely aerobic and the lactic acid content will be very low. . . ." "Animals driven to the slaughtering floor, or struggling violently during slaughter must have lost glycogan from their muscles."

About one-fifth of the birds, 19 out of 99, struggled when slaughtered. Sometimes the bird would be relaxed, then start to struggle during the bleeding period (1 minute), during the scalding (1 minute), or during picking. The time over which struggling or quivering occurred varied from 2 to 4.5 minutes, with the majority struggling (often intermittently) for about 3 minutes. The data obtained show that whether the results are compared within single groups or for all birds (disregarding aging time and massage treatments) struggling tended to hasten aging, in that tenderizing

of the muscles occurred more rapidly (Table 1) than in the birds that did not struggle. Since the number of birds that struggled during the experiment could not be controlled, the proportion of birds that did struggle should be given when results are stated.

SUMMARY

Roasters were held for 30 or 60 minutes after slaughter but before cooking was started. The 80 birds held for 30 minutes were divided into 7 groups; a control and the massaged groups. Massage was done by hand and by varying the picking time on two defeathering machines. The 19 birds held for 60 minutes were divided into a control and a hand massaged group.

The muscles of the machine-picked and hand-massaged groups of roasters of the 30-minute aged lot were scored more tender than those of the control birds (hand picked). The shear force values and the tenderness scores, however, did not show close agreement.

When the birds struggled during slaughter the muscles were scored more tender than muscles from birds of the same lot that did not struggle.

Roasters aged for 60 minutes after killing and before cooking was started were scored more tender than those held 30 minutes, the differences being significant at the 1% probability level.

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DETECTION OF CITRUS PULP IN NON-CITRUS JAMS

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In the commercial preparation of jams, the practice of mixing different fruit types is at times exercised in order to enhance the taste and flavor of the finished product, to supply pectin, fruit acids, and color which may be lacking in one fruit, or for the purpose of "stretching" an expensive fruit by a cheaper one.

The addition of citrus pulps to jams appears at present a problem specific to subtropical countries where such fruit grows abundantly and can be utilized advantageously as a raw material rich in pectin and fruit acids, and can be obtained at relatively cheaper prices.

The presence of various fruit types in a jam may be identified either chemically or microscopically. Thus Hinton *(6)* reported the chemical composition of a series of fruit jams in an attempt to detect adulteration by means of chemical analyses. Hanak *(4)* suggested the identification of carrots in jams by analyzing for its carotene contents, Guimaraes *(3)* recognized admixtures of other fruits to Guava jellies by the presence of starch or citric acid, since both compounds are not found in Guava jellies, while Muttelet *(8)* detected the presence of apples in certain jams by their characteristic proportion of aldoses to ketoses and Partridge *(10)* by the starch content.

Microscopic examinations of jams have been reported by various authors *(1, 2, 9)*. According to Harry *(5)*, the presence of date pulp may be recognized by the characteristic size and form of their cells. Two German publications *(7, 11)* described both chemical and microscopical methods of distinguishing between the use of fresh and dried plums in plum jams.

It was found that the major chemical constituents of citrus fruit did not differ sufficiently enough from those found in most other common, local fruit types to serve as reliable indicators of detection. The morphological structure of the cells, however, was found to differ conspicuously from the cells of other common fruit types, such as those of the plum and apricot.

The tissues of the citrus juice sacks consist of three well-defined layers *(12)*: an elongated epidermis, a transverse sub-epidermis, and a central parenchyma layer. These tissues were not found to be well suited for ready differentiation from other fruit cells. The white albedo layer of the citrus peel, however, is built of cells having a specific, angular, irregular shape, which are very readily differentiated from other fruit cells. The albedo was found to consist largely of these spongy parenchyma cells. Cells of identical shape also occur in the tissues enveloping the citrus segments and

in the tissues of the core. This spongy parenchyma forms an intricate network of elongated, branched, and very irregular cells which appear to be specific for all citrus species. Such cells are not found in either apricot or plum pulps. Figure 1 shows the characteristic cell structure found in orange pulp after it had been boiled for ¾ hour, sieved, and stored for 4 months.

Since these specific parenchyma cells are found in all parts of the fruit, they will appear in abundance in all citrus pulps, but less so in products in which citrus juice, rather than citrus pulp had been added. But even in orange juice about 30 such parenchyma cells were counted in a single drop of hand-reamed juice. These cells are very distinct and

FIGURE 1. Appearance of the parenchyma cells in cooked orange pulp.

large, so that no special staining is required. The method is equally applicable to other citrus species since lemon and grapefruit have been found to contain cells of similar shape.

While this method seems very convenient for qualitative determinations, there would even seem a possibility of its application for semi-quantitative work. In reamed juice the number of parenchyma cells was found to vary somewhat according to the method of reaming employed. It was found to be higher in juice reamed with automatic machinery, such as that of the American FMC type, than in that reamed with rotating reamers. Furthermore, there will be an increase in the number of cells with progressive maturation and there may, also, exist some varietal differences. The number of parenchyma cells will be far higher in products made from the entire fruit pulp, such as is commonly used in the manufacture of jams.

Such commercial citrus pulp which is prepared by subdividing the entire or the rasped fruit, boiling it with water and sieving, was found to contain about 25 times more parenchyma cells than the reamed juice.

Repeated microscopic counts of diluted samples showed that the proportion of citrus pulp in mixed jams can be estimated within about 10%. When mixtures of orange pulp and apricot or plum pulp were prepared in the laboratory, the relative count of typical fruit cells in such mixtures was found to approximate the true proportion of the two pulps rather closely.

SUMMARY

A simple qualitative method is reported for the microscopic detection of citrus pulp in fruit jams, as tested in apricot and plum jams. This method may lend itself to semi-quantitative work.

Acknowledgment

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BOTULINUM TOXIN FORMATION IN LIVER SAUSAGE[a]

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Even though preserved meats have been responsible for most of the outbreaks of botulism in Europe *(1)*, these products seldom are accountable for the disease in America. Sausages have been incriminated in 11 of the 37 outbreaks traceable to all meat products in this country in the past 50 years, but with two exceptions these were home preserved products *(7)*.

The fact that *Clostridium parabotulinum* has never been isolated from food or equipment in federally inspected meat packing houses *(5)* may help to explain why commercially prepared liver sausage has never been involved in an outbreak of botulism. Even so, in view of the wide distribution of spores of this organism *(1)* it must be assumed that they may sometimes get into the finished product but fail to grow and produce toxin under the usual conditions of manufacture and handling. That liver sausage is not a good medium for *C. parabotulinum* is shown by the work of Jensen *(6)*.

The natural casings that have long been used for sausages allow fairly rapid passage of oxygen and water. They also support growth of molds. Liver sausage packaged in these casings has a fairly short storage life at best, and if it is held at a temperature high enough for growth of *C. parabotulinum*, it spoils very quickly. With some of the artificial casings now in use, however, greatly extended storage times are practicable, even at temperatures higher than those ordinarily used *(8)*. This fact has led some sausage makers to fear that consumers and possibly retail merchants may neglect to refrigerate liver sausage properly and thus perhaps permit growth of toxin-forming organisms.

Little work dealing with the effect of plastic casings on growth of obligate anaerobes has been reported. Hall *(4)* showed that these organisms would not grow on the surface of agar covered with "Visking" film,[c] but this does not necessarily mean that they would fail to grow within a mass of sausage cased in Visking. The low permeability of Saran[d] indicates that this plastic film might permit growth of obligate anaerobes.

The purpose of the work reported here was to see whether spores of *C. parabotulinum* could germinate and produce toxin in liver sausage and, if so, to observe the effect of different factors on toxin formation in this product cased in Saran. The results of studies related to this have been reviewed by Jensen *(5)*.

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[b] Present address: Research and Product Development, Chain Belt Company, Milwaukee, Wisconsin.

[c] Visking is the name given to certain products of the Visking Corp., Chicago, Ill.

[d] Saran is made by the Dow Chemical Co., Midland, Michigan.

METHODS

Production of spores. A single strain of *C. parabotulinum*[*] was used in all experiments. The organism was designated "Hall A" strain and produced type A toxin. One liter of the medium used for spore production contained 5 g. of Difco tryptone, 5 g. of K_2HPO_4, 2.5 g. of glucose, 600 ml. of distilled water, and 400 ml. of liver extract prepared by the method of Garey, Foster, and Frazier *(2)*. After the reaction was adjusted to pH 7.6 with NaOH, the medium was dispensed in 100-ml. quantities in 6-ounce bottles and sterilized at 121°C.(250°F.) for 20 minutes.

The medium was inoculated with 1% of an active 18-hour culture of the organism and incubated at 37°C.(98.6°F.) for 1 week. The cultures were pasteurized at 80°C. (176°F.) for 10 minutes and the spores were recovered in a Sharples supercentrifuge. The spores were washed with sterile distilled water several times to remove adsorbed toxin and finally suspended in a small volume of sterile water. The number of viable spores in the suspension was estimated by plating in BBL (Brewer's modified) thioglycollate medium containing 1.5% agar. Plates were incubated anaerobically at 37°C. (98.6°F.) for 5 days.

Preparation of sausage. Published formulas for liver sausage *(3, 9)* call for a mixture of meats, much of which consists of pork livers, a mixture of spices, and salt. In addition sodium nitrate, sodium nitrite, sugar, milk powder, and perhaps other substances may be included. The exact composition of the product and methods of cooking and handling vary with different sausage makers.

The liver sausage used in this work was made by a method similar to that described in "Sausage and Meat Specialties" *(9)*. In the usual mixture, 2.5% salt, 0.1% sodium nitrate, spices, and a small amount of sugar were added to the meat. This product is referred to hereafter as the basal mixture. In some of the experiments sodium nitrite was added in place of or in addition to the sodium nitrate. The moisture content of the sausage was 44-47% and the reaction was pH 6.1-6.5.

Unless otherwise indicated in a later section, the sausage batter was inoculated with 5,000 spores of *C. parabotulinum* per g., stuffed into Saran casings in approximately 10-ounce units, cooked at 70°C.(158°F.) for 70 minutes, and incubated at 30°C. (86°F.). Samples were removed at intervals and tested for toxin.

Testing for botulinum toxin. Ten g. of sausage was mixed with 20 ml. of water and filtered through paper. Two mice were injected intraperitoneally with 0.5 ml. of the filtrate while a control mouse was injected similarly with a like amount of the filtrate that had been heated for 15 minutes in flowing steam. Botulinum toxin was considered to be present if the control mouse lived while both of those receiving the unheated filtrate died within 4 days with symptoms typical of botulism. Unheated filtrate of uninoculated sausage or of that inoculated with very small numbers of spores (Table 4) was never toxic, even after incubation at 30°C.(86°F.) for as long as 46 days. Therefore it is believed that the possibility of death caused by a nonspecific heat labile toxic substance is remote. Furthermore, in some of the experiments the cause of death was proved by protecting mice with specific antisera. Without exception, mice injected with heated filtrate from toxic sausage or protected with type A antiserum survived while those receiving unheated toxic filtrate with or without type B antiserum died.

RESULTS

The fact that liver sausage is a poor medium for *C. parabotulinum* was shown in several unsuccessful attempts to demonstrate toxin production in this product. Samples of commercial liver sausage containing sodium nitrate were obtained at a retail market, inoculated heavily with spores or vegetative cells, packed in test tubes and incubated anaerobically at 37°C.(98.6°F.) for as long as 11 days without toxin formation, although viable toxin-producing organisms were recovered at the end of the incubation period. Even when the inoculated sausage was mixed with an equal quantity of water to dilute the salt or when sodium thioglycollate was

[*] Furnished by Dr. Elizabeth McCoy.

added to counteract the oxidizing effect of the sodium nitrate, no toxin was detected after incubation for 11 days. When both water and sodium thioglycollate were added, however, toxin formed readily. Thus it appeared that inhibition was caused by both the salt and nitrate, either of which was able to prevent toxin formation under the conditions of the experiments.

To demonstrate further the effect of these substances, sausages were prepared from the basal mixture described earlier with the following variations: 1, both salt and nitrate omitted; 2, salt omitted; 3, nitrate omitted; and 4, neither omitted (the complete basal mixture). Portions of each mixture were prepared with and without spices, thus making a total of 8 combinations. Incubation was at 30°C.(86°F.). The first test for toxin was made at 9 days, at which time all sausages except the 2 lots containing both salt and nitrate were toxic. At the next test, 16 days, all sausages contained toxin. It is apparent that the combination of salt and nitrate delayed growth and toxin formation for 10 to 16 days, thus perhaps explaining earlier failures to demonstrate toxin formation in the commercial sausage when incubation periods of 4 to 11 days were used. When either salt or nitrate was omitted, toxin was formed rapidly. With the testing intervals used there was no demonstrable inhibitory effect of the spices.

Effect of salt concentration on toxin formation. After the above results had shown that the botulism organism could grow and produce toxin in liver sausage containing 2.5% salt and 0.1% sodium nitrate, several trials were run to compare the effect of 2.5, 3.0, and 3.5% salt on toxin formation. The concentration of sodium nitrate was kept constant at 0.1%. As can be seen in Table 1, Series 1, toxin formed in all sausages to which 2.5% salt had been added, although the time required for its detection ranged from 9 to 23 days. With 3.0% salt, one lot was toxic at 30 days while the other was not. None of the sausages containing 3.5% added salt became toxic within 30 days.

From these results it appears that 2.5% salt is near the critical level for toxin formation by *C. parabotulinum* in the presence of 0.1% sodium nitrate. Small variations in the salt content might therefore account for the observed differences in time required for toxin to form in the sausages to which 2.5% salt was added. Differences in the salt content of the original meat ingredients, differences in the water content of the finished batches, and small errors in weighing and mixing the added salt might account for the variations in time of toxin production. This explanation is supported by the results in Series 2 (Table 1) which show that an increase from 2.5% salt to only 2.75% prolonged the time for toxin detection from 6 to 16 days. An additional 0.25% (total 3.0) prevented toxin formation for over 23 days.

Effect of nitrate and nitrite on toxin formation. Since sodium nitrite can be added legally in low concentrations to cured meat products, its effect on toxin formation was tested. In the first experiment it was tried in addition to and in place of the sodium nitrate in the basal mixture, with the results shown in Table 2.

When added at the maximum allowable level (200 p.p.m.) sodium nitrite delayed toxin formation for a longer time than did 0.1% sodium nitrate; furthermore, the combination of nitrate and nitrite was more effec-

TABLE 1

Effect of salt concentration on toxin formation in liver sausage held at 30°C.(86°F.)[a]

SERIES 1

Salt added	Trial No.	Toxin + or − at indicated days			
		9	16	23	30
%					
2.50	1	−	+	+	+
2.50	2	−	+	+	+
2.50	3	−	−	+	+
2.50	4	+	+	+	+
2.50	5	−	−	+	+
3.00	3	−	−	−	−
3.00	4	−	−	−	+
3.50	2	−	−	−	−
3.50	3	−	−	−	−
3.50	4	−	−	−	−

SERIES 2

Salt added	Trial No.	Toxin + or − at indicated days					
		3	6	8	16	23	30
%							
2.00	6	+	+	+			
2.25	6	+	+	+			
2.50	6		+	+			
2.75	6			−	+	+	
3.00	6			−	−	−	
3.25	6			−	−	−	−

[a] In addition to salt the basal mixture contained 0.1% sodium nitrate. Sausage batter inoculated with 5,000 spores per g.

tive than was either compound alone. In further experiments with lower levels of nitrite it was necessary to add at least 100-150 p.p.m. to demonstrate inhibition of toxin formation beyond that observed in the basal mixture. Samples containing an initial concentration of 200 p.p.m. sodium nitrite with no nitrate or salt became toxic within 4 days.

Effect of temperature on toxin formation. The temperature of incubation used in most of these experiments, 30°C.(86°F.), is much higher than any that might be encountered under ordinary conditions of handling meat. It was selected as the extreme that might be met if no attempt were made

TABLE 2

Effect of nitrate and nitrite on toxin formation in liver sausage held at 30°C.(86°F.)[a]

Materials added to meat base[b]	Toxin + or − at indicated days			
	9	16	23	30
Salt plus nitrate (basal mixture)	−	+	+	+
Salt plus nitrite	−	−	−	+
Salt plus nitrate plus nitrite	−	−	−	−

[a] Sausage batter inoculated with 5,000 spores per g.
[b] Concentrations of added materials: salt, 2.5%; sodium nitrate, 0.1%; and sodium nitrite, 200 p.p.m.

at refrigeration. Table 3 shows the results obtained when sausages were stored at 30, 25, 18, and 10°C.(86, 77, 64.4, and 50°F.). As can be seen, a decrease of only 5°C.(9°F.) below the usual 30°C.(86°F.) almost tripled the time required for toxin to form. At the lower temperatures there was no toxin for the duration of the tests.

TABLE 3

Effect of temperature of incubation on toxin formation in liver sausage[1]

Temperature of incubation	Toxin first detected at
	days
30°C.(86°F.)	20
25°C.(77°F.)	58
18°C.(64.4°F.)	None in 90[2]
10°C.(50°F.)	None in 205[2]

[1] Basal mixture inoculated with 5,000 spores per g.
[2] All samples used up before toxin was detected.

Effect of number of spores on toxin formation. As far as is known, there is no available information on the numbers of spores of *C. parabotulinum* in meat products. The figure of 5,000 spores per g. used in most of these experiments was chosen arbitrarily with the idea of selecting an inoculum that was not unreasonably high yet one that would initiate growth when conditions permitted. To determine the effect of different numbers of spores on the time required for toxin to form, portions of the basal liver sausage mixture were inoculated with dilutions of a spore suspension calculated to provide 13,000, 1,300, 130, and 13 spores per g. respectively. Part of each lot was incubated at 30°C.(86°F.) and the remainder at 23°C.(73.4°F.). Table 4 shows the results for the samples incubated at the higher temperature. As might be expected, the number of spores had a marked effect on the time required for toxin to form.

TABLE 4

Effect of number of spores on toxin formation in liver sausage[1] held at 30°C.(86°F.)

Number of spores per g.	Toxin first detected at
	days
13,000	16
1,300	32
130	None in 46[2]
13	None in 46[2]

[1] Basal mixture.
[2] All samples used up before toxin was detected.

The sausages incubated at 23°C.(73.4°F.) were free of toxin at 60 days, at which time all samples had been used up. This result confirms those in Table 3, which showed that a reduction of the incubation temperature by only a few degrees greatly delays or prevents toxin formation.

Effect of casing on toxin formation. After it had been shown repeatedly that *C. parabotulinum* could grow and produce toxin in the basal liver sausage mixture stuffed in Saran it was natural to wonder whether the

same result would be obtained if the product were in a natural casing. To answer this question, sausage of the usual composition was inoculated with 5,000 spores per g.; then portions were stuffed into natural casings and into Saran, cooked as usual, and stored at 30°C.(86°F.). The sausages in Saran became toxic in 16 days while those in the natural casings were not toxic when the last test was made at 23 days.

The failure of the botulinum organism to form toxin in the product in natural casing can be explained by an increase in the salt concentration during incubation. The sausages were held in a fairly dry atmosphere and those in natural casings became badly dehydrated, while the ones in the less permeable Saran lost little or no moisture. Further evidence to support this explanation is found in results of unreported experiments with commercial Cleveland bolognas stuffed in Visking.[m] When this product was injected with spores of *C. parabotulinum* and incubated in a container to prevent drying, toxin formed readily. When it was incubated in a dry atmosphere, however, it showed evident shrinkage from loss of moisture and failed to become toxic.

That the natural casings per se did not inhibit toxin formation is shown by the fact that sausages containing nitrate but no salt became toxic quickly regardless of the casing material used.

Appearance of sausages incubated at 30°C.(86°F). Liver sausages prepared in porous casings lose moisture rapidly and develop color defects as a result of absorption of oxygen. Such evidence of spoilage is relatively easy to detect. Similar sausages stuffed in Saran, however, often showed insignificant signs of spoilage even after extended storage at warm temperatures. Even though some of these developed considerable gas, others did not and rarely was there a noticeable change in color of the sausage.

The appearance of gas in the sausage without evident shrinkage and color changes is of doubtful value as an indication of spoilage and possible growth of the botulinum organism. To illustrate this, samples of inoculated and incubated liver sausage with amounts of gas varying from none to very much were shown to 22 individuals with the question, "which if any of these would you eat?" None of the sausages was abnormal in color. Some contained toxin while others did not. Some of the sausages were refrigerated overnight to contract the gas and thus cause the casings to wrinkle, while others were left warm with tight casings. No sausage was rejected by everyone and none was accepted by everyone. Sausages with wrinkled casings were rejected more frequently than were those with tight casings. Slight amounts of gas were not detected by most persons. Perhaps by coincidence, the sausage that was acceptable to the greatest number of individuals contained botulinum toxin.

DISCUSSION

The results reported here demonstrate that *C. parabotulinum*, as exemplified by the single strain of type A used in this study, can produce toxin in liver sausage only if a large number of spores is present and the sausage is incubated for an extended period of time at a warm tempera-

[m] Regenerated cellulose film.

ture. It is unlikely that the conditions necessary for toxin formation would ever be met in commercial handling of this product. Sausage makers are well aware of the means of preventing any danger of botulism from liver sausage and our results merely emphasize the importance of well known practices of sanitation and refrigeration.

Unfortunately there is no way to tell with certainty by visual examination whether *C. parabotulinum* has grown in liver sausage. Since this organism is putrefactive and produces gas it might be thought that odor or appearance of the sausage would indicate to a consumer that something was wrong with the product. These indicators are not reliable. The aroma of the spices normally in liver sausage tends to mask slight undesirable odors that may be formed and the development of amounts of gas detectable by the consumer is inconsistent.

Stuffing liver sausage in relatively impervious casing materials, such as Saran, introduces a possibility of botulinum intoxication if the product is grossly mishandled. This does not in any way mean that the less permeable casings should be avoided. The danger of botulism from this product can be averted easily for an indefinite time by insuring an adequate concentration of the preservative ingredients and by keeping the product refrigerated constantly before consumption.

SUMMARY

Liver sausage batter was inoculated with spores of *Clostridium parabotulinum*, type A, stuffed in Saran and incubated at 30°C.(86°F.). When the sausage contained 2.5% salt, 0.1% sodium nitrate, and 5,000 spores per g., toxin was formed in 9 to 23 days. This time was markedly increased by a slight increase in the salt concentration, a decrease in the temperature of incubation, or a decrease in the number of spores. The addition of 100-200 p.p.m. sodium nitrite to the sausage enhanced the inhibitory effect of salt and nitrate. Sausages inoculated with 5,000 spores per g. and held at 18°C.(64.4°F.) or 10°C.(50°F.) did not become toxic in 90 and 205 days respectively. Thus it would appear that there is no practical likelihood of botulinum intoxication from commercial liver sausage under commercial conditions of preparation and proper storage prior to use.

The inoculated liver sausage usually retained an acceptable color but showed variable amounts of gas when held for as long as 4 weeks at 30°C.(86°F.). There was no correlation between the appearance of visible amounts of gas and the presence of toxin.

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EFFECT OF FROZEN STORAGE AND SUBSEQUENT COOKING ON THE THIAMINE, RIBOFLAVIN, AND NICOTINIC ACID CONTENT OF PORK CHOPS[a]

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Many workers have reported that pork is an excellent source of thiamine for the human dietary. Hughes *(5)*, Miller, Pence, Dutcher, Ziegler, and McCarty *(10)*, Ensminger, Heinemann, Cunha, and McCulloch *(3, 4)*, and Pence, Miller, Dutcher, and Ziegler *(11)* have studied the pig's ability to store thiamine. Ensminger and coworkers *(3)* and Heinemann et al. *(4)* have also investigated the riboflavin storing ability of the pig. These workers have found that the dietary intake of thiamine and riboflavin has a direct bearing on the content of these vitamins in the muscle. Little work has been done on the effect of nicotinic acid intake and its deposition in pork tissue. Waisman and Elvehjem *(16)* reviewed early vitamin work with meats.

Dann and Handler *(2)*, in their study of 14 samples of fresh pork muscle, found the mean nicotinic acid content was 61 $\pm$ 5.3 μg. with a range of 41-102 μg. per g. of fresh tissue. These workers reported a loss of 19-57% of nicotinic acid from pork chops during frying.

McIntire, Schweigert, Henderson, and Elvehjem *(7)* report that thiamine values range from 7.4-15.2 μg. per g. of fresh pork loin; and riboflavin values range from 1.6-3.0 μg. per g. The nicotinic acid values were more constant, ranging from 31-49 μg. per g. of the fresh tissue, most of the samples containing about 35 μg. per g. The average retention of these vitamins during cooking was: 70% for thiamine after roasting and broiling, and 50% after braising; 85% for nicotinic acid after roasting and broiling, and 65% after braising; and 85% of riboflavin as a result of any of the cooking methods. Schweigert, McIntire, and Elvehjem *(13)*, in a study of fresh and cured hams, reported that the thiamine, riboflavin, and nicotinic acid content varied from 4.7-9.9, 1.9-2.9, and 26-52 μg. per g. of tissue, respectively. These workers found the average retention of vitamins after 14 days of storage at —4°C. was 92 % for the thiamine and nicotinic acid and 85% for the riboflavin. The average retention after roasting was 58% for thiamine, 79% for the nicotinic acid, and 74% for riboflavin; after frying, 86% for thiamine, 85% for the nicotinic acid, and 77% for the riboflavin.

The thiamine and riboflavin content of cooked pork loins was found by Brady, Peterson, and Shaw *(1)* to be 70 to 80% of that in uncooked loin muscles when calculated on a dry, fat-free basis. Rice, Fried, and Hess

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(12) found variation in the vitamin retention of pork loins stored at 4°C. for as long as 68 days. There was a loss of less than 10% of the thiamine and riboflavin, and as much as 54% loss of niacin.

The literature is lacking information on the effect of frozen storage on the retention of vitamins. With the advent of increased usage of freezer storage of meats, it was thought desirable to undertake a study on the effect of freezing, thawing, and cooking on the thiamine, riboflavin, and nicotinic acid content of pork chops.

EXPERIMENTAL PROCEDURE

Pork loins from hogs of comparable nutritional background, weighing 200-210 pounds live weight, were used in this study. The Poland China barrows were bred and fed, and the carcasses processed by the Experiment Station.

Within 24 hours after slaughter the left loins were removed from the carcasses between the third and fourth ribs, and cut into uniform size chops ¾ inches in thickness. Each chop was individually tagged and processed according to the schedule presented in Table 1. The chops that were to be frozen were first wrapped in double-treated waxed freezer paper and then in regular heavy butcher paper. Chops placed in the same package were separated from each other by freezer paper prior to wrapping.

TABLE 1

Treatment of pork chops

Package number	Pork Loins						Freezing treatment[d]	Storage period	Treatment at time of analysis
	A	B	C	D	E	F			
	Chops[c]								
I	1	17	14	11	8	5	Not frozen	None	Raw
II	2	18	15	12	9	6	Quick frozen	24 hrs.	Thawed
III	3	1	16	13	10	7	Slow frozen	48 hrs.	Thawed
IV	4	2	17	14	11	8	Quick frozen	3 mos.	Thawed
V	5	3	18	15	12	9	Slow frozen	3 mos.	Thawed
VI	6	4	1	16	13	10	Quick frozen	3 mos.	Cooked frozen
VII	7	5	2	17	14	11	Slow frozen	3 mos.	Cooked frozen
VIII	8	6	3	18	15	12	Quick frozen	3 mos.	Thawed-cooked
IX	9	7	4	1	16	13	Slow frozen	3 mos.	Thawed-cooked
X	10	8	5	2	17	14	Quick frozen	6 mos.	Cooked frozen
XI	11	9	6	3	18	15	Slow frozen	6 mos.	Cooked frozen
XII	12	10	7	4	1	16	Quick frozen	6 mos.	Thawed-cooked
XIII	13	11	8	5	2	17	Slow frozen	6 mos.	Thawed-cooked
XIV	14	12	9	6	3	18	Quick frozen	6 mos.	Thawed
XV	15	13	10	7	4	1	Slow frozen	6 mos.	Thawed
XVI	16	14	11	8	5	2	Quick frozen	48 hrs.	Thawed
XVII	17	15	12	9	6	3	Slow frozen	24 hrs.	Thawed
XVIII	18	16	13	10	7	4	Not frozen	None	Cooked

[c] Chop number refers to location from which the chop was cut beginning from the rib end.
[d] Slow frozen means 0°F.(−18°C.) and quick frozen means −15°F.(−26°C.).

The wrapped pork chops were stored at two temperatures. Those indicated as "slow frozen" (S.F.) were frozen and stored at the generally recommended freezer storage temperature of 0°F.(−18°C.). The chops listed as "quick frozen" (Q.F.) were frozen and stored at a temperature of −15°F.(−26°C.).

Where indicated in Table 1, the "thawed" (T.) chops were thawed at room temperature [70°F.(21°C.)] and those "cooked" were either cooked in a frozen state or thawed at room temperature and then pan fried (T.C.) as recommended by the National Livestock and Meat Board *(8)*.

In all vitamin determinations the longissimus dorsi muscle was used for analysis. The muscle was cut out of the chop, the fat was trimmed off, and then the muscle was finely ground.

Thiamine was determined by a modification of the thiochrome method as described by Michelsen, Condiff, and Keyes *(9)*; riboflavin was measured microbiologically by the method of Snell and Strong *(14)*; and nicotinic acid by the method of Snell and Wright *(15)*. Duplicate determinations were made on all samples. Results are reported on an oven-dry basis (95°C.).

RESULTS AND DISCUSSION

The means of duplicate determinations for the thiamine, riboflavin, and nicotinic acid content in pork chops from each of the six loins and for each of the 18 treatments are given in Table 2. The loin and treatment

TABLE 3

Analysis of variance (mean squares only) in the thiamine, riboflavin, and nicotinic acid content of pork chops

Source of variation	Degrees of freedom	Thiamine	Riboflavin	Nicotinic acid
Total	215	104.6	2.13	3,265
Loins	5	202.3*	1.01	5,127
Treatments	17	814.4**	14.70**	24,289**
Raw vs. cooked	1	2,318.7**	19.26**	10,417*
24 hours vs. 48 hours	1	151.9	5.95*	588
24 Hours				
Raw vs. quick frozen	1	254.2*	3.38	6,112
Raw vs. slow frozen	1	339.8*	4.59	17,713*
Quick frozen vs. slow frozen	1	6.2	0.20	3,060
48 Hours				
Raw vs. quick frozen	1	85.2	1.35	12,105*
Raw vs. slow frozen	1	196.6	4.00	18,482**
Quick frozen vs. slow frozen	1	123.3	3.23	672
3 Months vs. 6 Months	1	578.4**	0.81	3,803
3 Months				
Quick frozen vs. slow frozen	1	70.8	0.42	840
6 Months				
Quick frozen vs. slow frozen	1	71.6	0.03	470
3 Months and 6 Months				
Thawed vs. thawed-cooked	1	5,075.0**	25.22**	161,950**
Thawed vs. cooked-frozen	1	2,126.3**	5.85*	231,968**
Thawed-cooked vs. cooked-frozen	1	631.4**	6.77*	6,273
Loins × treatment	85	60.7**	1.30**	2,616**
Error	108	22.9	0.43	381

* Indicates significance at the 0.05 level of probability.
** Indicates significance at the 0.01 level of probability.

means, over all 18 treatments and all 6 loins, respectively, are given in the last column and at the bottom of the table. Certain grouped treatment means are also presented at the bottom of the table for more accurate comparisons of the different kinds of treatments.

The significance of differences among the means given in Table 2 was determined by the analysis of variance. The mean squares from the analy-

sis of variance for each of the three vitamins are presented in Table 3. Differences between several individual treatment means and combinations of treatment means were tested for significance. The set of comparisons made do not constitute an orthogonal set. However, since they were chosen from the type of treatment applied rather than from the means observed, they are valid tests of significance.

TABLE 4

Percentage loss of thiamine, riboflavin, and nicotinic acid due to cooking

Vitamin	Freshly cooked	Averages of samples after 3 and 6 months of storage	
		Thawed and cooked	Cooked frozen
	Loss,%	*Loss,%*	*Loss,%*
Thiamine	56	54	35
Riboflavin	34	27	13
Nicotinic acid	17	36	43

Differences between the loin means in the last column of Table 2 were significant for thiamine only. The average content of thiamine (over all 18 treatments) varied from 19.6 to 25.0 μg. per g. of tissue. Considerable variation existed from one loin to another in nicotinic acid content but because of the large error term the differences were not significant at the 0.05 level. The differences from one loin to another suggest that genetic and, in addition, permanent environmental differences may exist between pigs in their ability to store thiamine and nicotinic acid.

Differences between the 18 treatments were highly significant for each of the three vitamins. However, differences between treatments were not in the same ratio for all of the six loins as shown by the highly significant loin $\times$ treatment interaction of all three vitamins. An example of this interaction is the variation in the percentage loss of these vitamins from fresh and raw to cooked samples. The loss of thiamine from one loin to another varied from 40 to 66%, riboflavin from 5 to 83%, and nicotinic acid from 5 to 34%.

There are at least three possible explanations for the loin $\times$ treatment interaction. One is the possibility that the plus and minus deviations among the errors were correlated between the two determinations on the same sample. The effect of such correlation among the errors would be to make the error mean square too small and the loin $\times$ treatment mean square too large. This could have occurred if differences between determinations made by different men and at different times are important, since these determinations were made by three different men and the determinations from one treatment to another had to be made at different times. A second possibility is that of genetic or permanent environmental differences between the loins in their ability to retain their vitamin content under the various treatments. A third, and perhaps most important, explanation is the possibility of different amounts of these three vitamins from one end of the longissimus dorsi muscle to the other. If this were true, a loin $\times$ treatment

interaction would result, since it was impossible for each of the 18 treatments to receive a chop from the same section of the loin.

The percentage loss of thiamine, riboflavin, and nicotinic acid due to cooking is summarized in Table 4. These reductions in vitamin content were significant or highly significant in every case, as shown in Table 3. Cooking the pork chops that had been stored for 3 and 6 months in the frozen state resulted in a saving of 19% of the thiamine and 14% of the riboflavin as compared to thawing and then cooking. These differences were significant beyond the 0.01 and the 0.05 levels of probability, respectively. On the other hand, the loss of nicotinic acid increased when the chops were cooked in the frozen state. However, this difference is not statistically significant, indicating that there is probably no difference in the retention of nicotinic acid when pork chops are cooked by these two methods.

Differences in the thiamine or nicotinic acid content from 24 to 48 hours were not significant. The reduction of 0.7 μg. per g. of tissue in riboflavin from 24 to 48 hours of frozen storage was significant. Those chops that were frozen and stored for 24 hours had significantly less thiamine than the fresh raw chops. The nicotinic acid content decreased significantly (from 248 to 194 μg. per g. of tissue) in chops which were kept at 0°F. (−18°C.) (S.F.) for 24 hours.

There was a 21% reduction in thiamine content after 3 months of frozen storage and an additional loss of 19% after 6 months of frozen storage. Both of these reductions were highly significant statistically. A highly significant loss of 31% also occurred in the riboflavin content of chops after 3 months of frozen storage. There was no additional loss of riboflavin from 3 to 6 months of storage. Only a slight insignificant drop occurred in the nicotinic acid content during frozen storage up to 6 months. These results are contrary to those obtained with lamb chops and reported in a previous paper *(6)*. More niacin was found in freezer stored lamb chops than in the fresh chops, indicating a possible synthesis of this vitamin during storage.

No significant differences occurred in the thiamine, riboflavin, or nicotinic acid content between pork chops that were kept at −15°F.(−26°C.) (Q.F.) and those that were kept at 0°F.(−18°C.) (S.F.). In general, the "quick frozen" chops contained slightly more of each of these vitamins than did the "slow frozen" chops that were stored for a comparable length of time. However, none of these differences were statistically significant because of the large error terms.

SUMMARY

Pork chops from six Poland China hogs were subjected to 18 different treatments and the thiamine, riboflavin, and nicotinic acid content was determined. The chops were stored at either −15°F. or 0°F.(−26°C. or −18°C.) for 24 hours, 48 hours, 3 months, and 6 months. After freezer storage the chops were cooked in the thawed state and also in the frozen state.

The six loins differed significantly in thiamine content but not in the other two vitamins studied. More thiamine, riboflavin, and nicotinic acid

TABLE 2

Mean content of thiamine, riboflavin, and nicotinic acid (in micrograms per g. of tissue) in the longissimus dorsi muscle of pork chops from six loins*

	Fresh		Frozen														Loin means
			24 Hours Thawed and analyzed		48 Hours Thawed and analyzed		3 Months						6 Months				
							Q.F.			S.F.			Q.F.			S.F.		
	Raw	Cooked	Q.F.	S.F.	Q.F.	S.F.	T.	T.C.	C.F.	T.	T.C.	C.F.	T.	T.C.	C.F.	T.	T.C.	C.F.	
Loin A																			
Thiamine	32.0	18.0	28.4	30.8	32.2	25.9	22.8	15.1	17.8	23.1	15.2	29.6	28.5	12.1	17.3	26.1	10.1	19.4	22.5
Riboflavin	6.0	1.0	6.2	6.9	6.0	4.7	3.8	2.3	3.0	3.4	3.4	3.0	4.4	2.8	3.4	4.2	2.7	3.5	3.9
Nicotinic acid	214.0	176.0	190.0	209.0	192.0	158.0	227.0	147.0	122.0	184.0	124.0	180.0	241.0	183.0	114.0	253.0	134.0	159.0	178.0
Loin B																			
Thiamine	33.9	13.3	29.0	35.0	28.8	20.9	18.6	9.6	10.6	18.2	12.0	20.4	26.6	13.5	15.0	22.4	11.5	14.1	19.6
Riboflavin	6.2	5.0	5.6	8.2	6.2	6.2	3.6	2.2	2.4	3.5	2.6	2.6	3.2	2.4	2.6	3.6	2.2	2.5	3.9
Nicotinic acid	264.0	243.0	253.0	234.0	252.0	170.0	188.0	182.0	152.0	152.0	208.0	166.0	304.0	89.0	103.0	300.0	168.0	138.0	198.0
Loin C																			
Thiamine	45.4	18.1	33.2	33.4	42.4	41.8	38.0	8.8	13.2	35.0	9.9	20.4	29.2	13.2	9.6	34.0	11.0	12.6	25.0
Riboflavin	5.6	4.0	5.5	5.6	5.8	5.2	4.1	2.6	3.2	2.8	2.8	3.6	3.4	2.4	2.3	3.6	2.0	2.7	3.7
Nicotinic acid	244.0	205.0	161.0	184.0	178.0	166.0	266.0	116.0	84.0	172.0	114.0	120.0	240.0	132.0	136.0	256.0	132.0	136.0	169.0
Loin D																			
Thiamine	31.9	19.1	25.1	26.4	44.4	29.8	23.9	12.8	18.4	33.2	9.1	24.9	15.4	12.2	7.7	19.9	6.6	8.2	20.5
Riboflavin	4.2	4.0	6.2	5.8	6.0	6.1	3.4	3.6	5.0	4.0	3.4	5.3	3.0	2.6	3.7	3.6	2.8	3.2	4.2
Nicotinic acid	300.0	198.0	260.0	222.0	216.0	234.0	254.0	178.0	88.0	240.0	90.0	134.0	229.0	136.0	104.0	230.0	127.0	60.0	183.0
Loin E																			
Thiamine	38.2	14.0	33.2	23.2	29.1	32.5	27.6	28.3	25.6	32.6	18.8	32.6	43.8	10.1	11.3	26.2	6.0	16.8	25.0
Riboflavin	4.6	3.6	6.4	4.4	4.8	4.6	4.8	3.5	3.6	3.2	3.3	3.9	6.5	2.2	2.5	2.7	3.7	4.1	4.0
Nicotinic acid	246.0	207.0	202.0	162.0	194.0	211.0	242.0	127.0	166.0	254.0	132.0	162.0	172.0	102.0	94.0	146.0	92.0	102.0	168.0
Loin F																			
Thiamine	29.0	9.8	22.4	16.2	26.0	25.0	32.2	22.0	16.9	26.8	9.2	26.6	31.0	15.4	14.0	15.0	8.8	21.4	20.4
Riboflavin	5.2	3.5	6.4	6.2	5.8	3.4	4.6	2.9	3.0	3.0	2.1	3.0	4.9	2.6	2.8	3.4	2.9	3.7	3.9
Nicotinic acid	222.0	210.0	232.0	152.0	190.0	218.0	208.0	221.0	184.0	260.0	166.0	168.0	268.0	224.0	77.0	150.0	141.0	128.0	190.0
Treatment means																			
Thiamine	35.0	15.4	28.5	27.5	33.8	29.3	27.2	16.1	17.1	28.2	12.4	25.8	29.1	12.8	12.5	23.9	9.0	15.4	22.2
Riboflavin	5.3	3.5	6.1	6.2	5.8	5.0	4.0	2.9	3.4	3.3	2.9	3.6	4.2	2.5	2.9	3.5	2.7	3.3	3.9
Nicotinic acid	248.0	207.0	216.0	194.0	203.0	193.0	231.0	162.0	132.0	210.0	139.0	155.0	242.0	144.0	105.0	223.0	132.0	121.0	181.0
Grouped treatment means																			
Thiamine			28.0		31.6		20.1			22.1			18.1			16.1			
Riboflavin			6.1		5.4		3.4			3.3			3.2			3.2			
Nicotinic acid			205.0		198.0		175.0			168.0			164.0			159.0			

*Q.F., S.F., T., T.C., and C.F. mean "quick frozen" (−15°F.), "slow frozen" (0°F.), thawed, thawed and cooked, and cooked-frozen, respectively.

was lost during storage and cooking from some loins than from others. Possible explanations for these results are discussed.

Cooking pork chops in the frozen rather than thawed state resulted in a saving of 19% of the thiamine and 14% of the riboflavin. No difference occurred in the amount of nicotinic acid lost by the two methods of cooking.

Freezer storage of pork chops for 6 months resulted in a loss of 40% of the original thiamine and 31% of the original riboflavin. No loss occurred in the nicotinic acid content during frozen storage. Only small insignificant differences were found between the quantity of these three vitamins in chops stored at −15°F.(−26°C.) and in those chops stored at 0°F. (−18°C.) for a comparable length of time.

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THE PREDOMINANCE OF MICROCOCCI IN THE FLORA OF EXPERIMENTAL FROZEN TURKEY MEAT STEAKS

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In their studies of the types of bacteria surviving in frozen vegetables, Lochhead and Jones *(1, 3, 4)* found that micrococci and species of *Flavobacterium* resist freezing to a larger extent than other types occurring in fresh-pack vegetables, and that they comprise a noticeably greater percentage of types surviving after nine months' freezing. They also found that of 50 types of micrococci isolated from frozen-pack vegetables, 12 strains produced an enterotoxic substance. Their findings direct attention to the possible importance of the *Micrococcus* group of organisms in relation to the proper handling of frozen vegetables.

In making aerobic counts and culture isolations from frozen turkey meat steaks, we also have found that most of the strains isolated belong to the *Micrococcus* genus. These aerobic counts and bacterial isolations were made from 12 turkey steaks during the experimental work on the freezing preservation of turkey meat steaks by Klose, Hanson, and Lineweaver *(2)*. These isolations were organisms present in the product at the end of the storage period.

PROCEDURE

Laboratory-prepared frozen turkey meat steaks were held at 0°F.(−17.8°C.) for 8 months and then thawed at room temperature (75°F., 24°C.) for 2 hours. Fifty g. were mixed with 450 g. of sterile water in a sterile blender jar for 1 minute and serial dilutions were plated out in duplicate on tryptone glucose extract agar. The plates were incubated at 90°F.(32°C.) for 48 hours. After the aerobic counts were made, culture isolations were made from the various plates of the different samples.

The colonies were selected for isolation from the entire surface of the various plates. Every colony that had, or seemed to have, a unique appearance when examined microscopically, including both surface and subsurface colonies, was selected. The cultures were tested for purity by plating and replating and were finally grown on slants of tryptone glucose extract agar. Culture identification was made according to the Manual of Methods for the Pure Culture Study of Bacteria *(5)* and Bergey's Manual *(6)*.

RESULTS

The aerobic plate counts of the turkey steaks were very low, ranging from 300 to 3,000 per gram.

Originally, 113 colonies were selected for isolation from surfaces of the various plates. From the colony description, streak appearance on tryptone glucose extract agar slants, morphology, and Gram stain, these 113 isolates were roughly divided into 23 groups. By rechecking, replating, and further testing, 57 of the isolates were eliminated as being duplicates from the same turkey steaks. The remaining 56 isolates were then examined in detail and

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identified as belonging to 16 species, representing 5 genera. Fifteen of these 56 were further eliminated as being duplicates from the same turkey steaks. The genera and species are listed below. The numbers in parentheses indicate the number of steaks from which the species were isolated: *Alcaligenes marshallii* (2), *Bacillus firmus* (2), *B. cereus* (1), *B. sphaericus* (1), *Flavobacterium marinum* (3), *Lactobacillus brevis* (2), *Micrococcus candidus* (7), *M. caseolyticus* (2), *M. conglomeratus* (1), *M. epidermidis* (3), *M. flavus* (6), *M. luteus* (5), *M. pyogenes var. albus* (2), *M. rhodochrous* (1), *M. urea* (2), and *M. varians* (1).

SUMMARY

From laboratory-prepared turkey meat steaks which had been frozen for eight months, 41 strains of aerobic bacteria were isolated, representing 5 genera. Most of the strains (31) belonged to the genus *Micrococcus* (as has been found by others for frozen vegetables).

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FACTORS INFLUENCING THE STABILITY OF THIAMINE DURING HEAT STERILIZATION[a]

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(Manuscript received May 22, 1951)

The effect of heat and certain environmental factors on thiamine stability has been the subject of several comprehensive investigations *(2, 3, 8, 9, 10, 12, 13, 14, 16, 17, 18, 19)*. There is good evidence that in pure thiamine solutions the destruction by heat is primarily a hydrolytic cleavage to give pyrimidine and thiazole derivatives *(20)*, and that the reaction rate may be expressed by the conventional equation for a first-order reaction. The effect of pH on the rate of thiamine destruction in pure solutions has been explored *(8, 16)*, with the result that it is well known that thiamine is less stable in alkaline than in acid solutions. Although the stability of thiamine in the presence of extraneous materials, such as foodstuffs, and the more common anions also has been investigated *(6, 17)*, conclusions from this work often have been masked by factors which are only vaguely understood.

An expanded knowledge of the factors which influence the stability of thiamine during the sterilization of canned foods is needed by those individuals engaged in developing new food processing methods which might improve the retention of this important vitamin. This is especially true if such information could lead to generalizations in regard to destruction rates at elevated temperatures. Recent descriptions *(5, 11, 15)* of new processing methods make reference to the markedly improved thiamine retentions which may be realized commercially through the use of high-temperature, short-time processing methods. Some workers *(5)* have pointed out that the amount of thiamine destruction during sterilization has been often used as an index to other chemical changes which take place during processing.

EXPERIMENTAL

Four vegetables which are normally processed in large quantities were selected for systematic investigation with regard to the effect of time, temperature, and other variables which might affect thiamine stability. For this purpose, tomato juice was chosen as a typically acid product which requires a relatively mild heat treatment in order to render it sterile. Peas and corn were selected as products which require a relatively intense heat treatment for sterilization, and which represent a high and a low thiamine product, respectively. Lima beans were included because significantly different degrees of retention of thiamine had been previously noted upon processing mature and immature Lima beans in the same retort (71 and 50%, respectively) *(4)*.

As a general approach, it was decided that each of these products should be processed over a rather wide temperature range, and that the thiamine content should be determined at relatively short intervals of time during the course of processing. In order to assure a uniform temperature over substantially all of the time interval of the experiment, all processing was done in small containers (211 x 214 or 208 x 006), and

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in most instances with the aid of agitation so as to render the come-up period a relatively small portion of the processing time. Thiamine determinations were made using the conventional thiochrome method *(1)*. All determinations were made in duplicate or triplicate.

Data from this type of experiment are most conveniently reported in graphic form on semi-log paper, with the percentage retention being plotted against the time during which the samples are subjected to the processing temperature. When plotted in this form, a straight line relationship indicates that the reaction is proceeding according to rule for a first-order reaction. This rule may be stated in either of two ways: first, the fractional decrease of the reactive component per unit of time is constant; or second, the rate of decrease of the active component is proportional to that component's concentration. Mathematically, either of these two statements may be expressed in differential form as

$$-(dc/C)/dt = K \qquad (1)$$

where $-(dc/C)$ is the fractional decrease of concentration C taking place during a time dt, and K is a constant often called the reaction rate constant. K is not independent of temperature.

Equation (1) may be integrated to give

$$2.3 \log C/C_o = K(t - t_o) \qquad (2)$$

where C_o is the concentration at a time t_o, and C is the concentration at a later time t.

The reaction rate constant K may be determined graphically from the slope of the curve obtained by plotting log C/C_o (or log of the percentage retention) vs. time; or by substituting experimental data directly into equation (2).

This particular method of handling the data has been used by previous workers who have demonstrated that the decomposition of thiamine under some, but not all, conditions proceeds according to a first-order reaction. For reactions which proceed according to the equation given above, it is a relatively simple matter to use the reaction rate constant K for investigating the effects of temperature, pH, etc. Unfortunately, as will be seen from the data in this report, it is only in special cases where the destruction of thiamine proceeds according to the equation. However, even when the reaction does not proceed in a first-order manner, it will be found convenient to discuss it in terms of its divergence from a first-order reaction.

RESULTS AND DISCUSSION

Figures 1, 2, 3, and 4 for peas, corn, tomato juice, and puréed Lima beans, respectively, present the behavior of thiamine in these products at elevated temperatures and at their normal pH. From these curves it is readily apparent that when the logarithm of the percentage retention is plotted against time, a linear relationship is shown for thiamine in peas, while in each of the three other products the curves are characterized by a large initial slope which decreases with respect to time. In subsequent work with peas, the linear relationship was shown for both vacuum and brine packed whole peas and for puréed peas. The addition of synthetic thiamine hydrochloride to the peas before processing altered neither the linearity nor the slope of the curves as shown in Figure 1. The K values obtained in the experiments with peas are shown in Table 1. Furthermore, as shown by Figure 5, in which the log of the reaction rate constant K for peas is plotted against the reciprocal of the absolute temperature, a straight line relationship is obtained. Using the reasoning applied by Evenden *(7)* to the oxidation of ascorbic acid, the straight line relationship of Figure 5 may be interpreted as evidence that the mechanism by which thiamine is lost in peas does not vary in its nature, but only in its rate over the temperature range studied [220-270°F.(104.5-132.5°C.)].

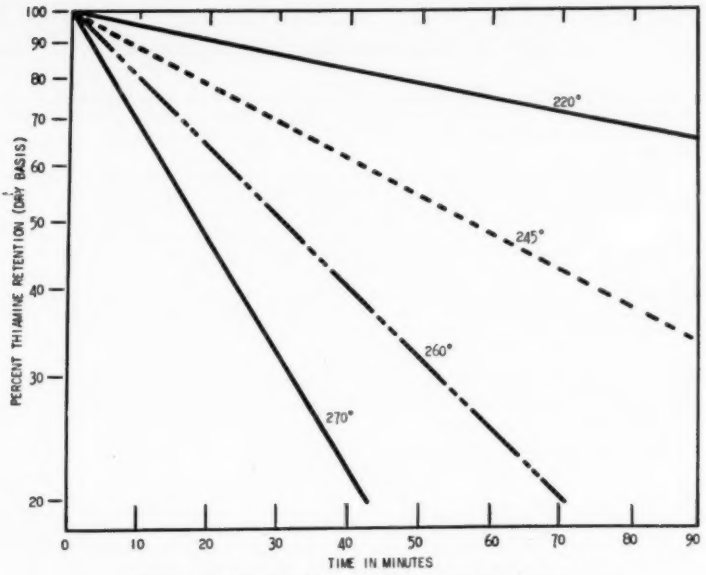

FIGURE 1. Thiamine stability during processing of peas.

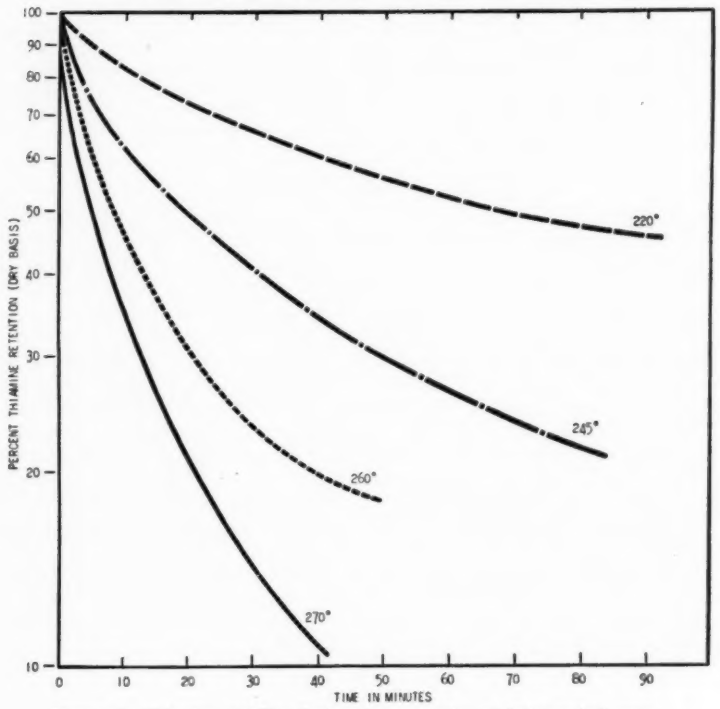

FIGURE 2. Thiamine stability during the processing of brine-packed corn.

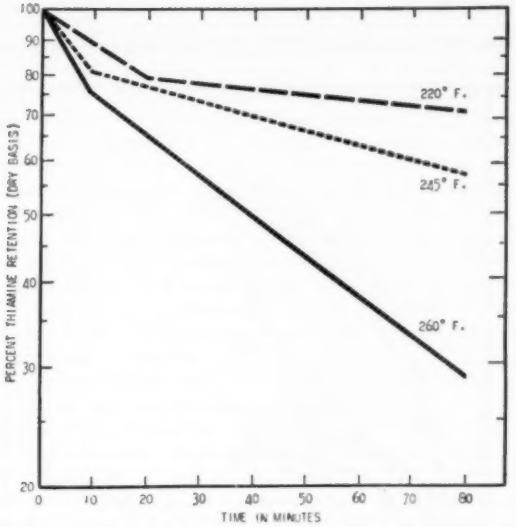

FIGURE 3. Thiamine stability during the processing of tomato juice.

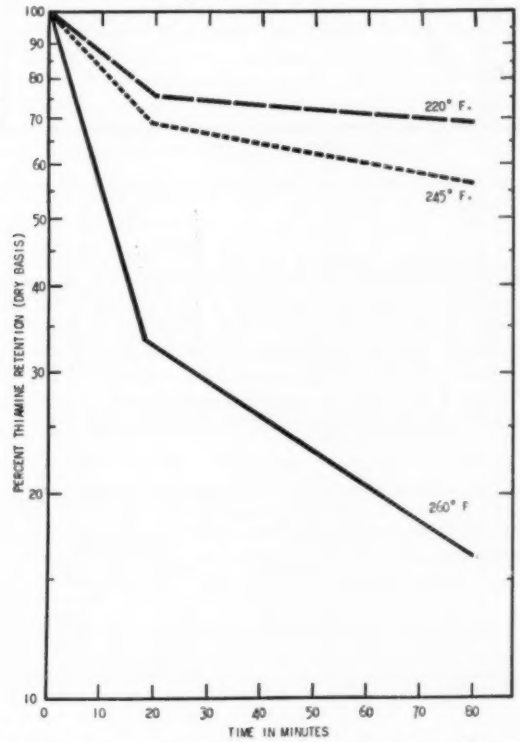

FIGURE 4. Thiamine stability during the processing of puréed Lima beans.

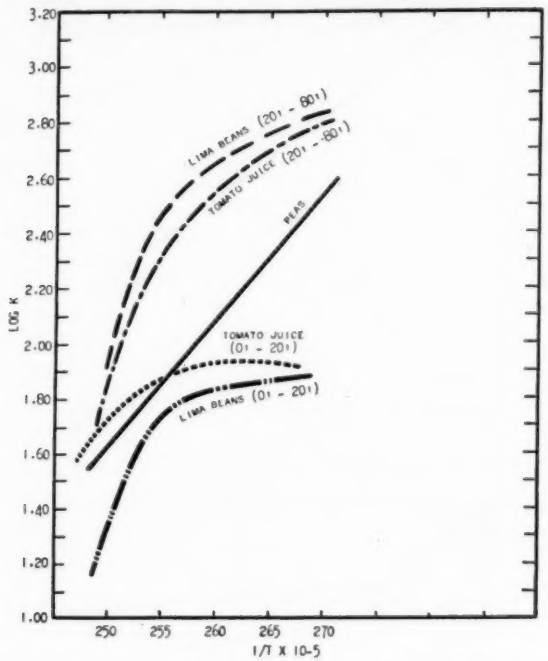

FIGURE 5. Influence of time and temperature on thiamine reaction rates during thermal processing.

Although a similar treatment of the data obtained for thiamine in tomato juice and Lima beans is not justified because of each of these products the requirements are not satisfied, a plot of log K of the straight portions of each of the curves against 1/T showed a marked non-linearity, thereby suggesting that the mechanism of loss in these products varies in both nature and rate, depending upon the conditions of the experiment.

From Figures 2, 3, and 4, it may be observed that, in general, the slope of the curves decreases during the course of processing. When viewed in

TABLE 1

Reaction rate constant K for the retention of thiamine in peas at various temperatures

Type of pack	Temperature			
	220°F.	245°F.	260°F.	270°F.
	K, *minutes*$^{-1}$	K, *minutes*$^{-1}$	K, *minutes*$^{-1}$	K, *minutes*$^{-1}$
Brine pack	0.0058	0.0122	0.0286	0.0351
Vacuum pack	0.0046	0.0142	0.0226	0.0351
Puréed pack	.....	0.0069	.....	.....
Puréed pack (phosphate buffer)	.....	0.0108	.....	.....

terms of the equation for a first-order reaction, this may be interpreted as a decreasing value of K, the fractional loss per unit of time. Two factors which might be conducive to a decreasing K have been postulated. First, since the reaction rate constant K is known to decrease with pH, a decreasing K might be expected in those products whose pH is lowered as a result of thermal processing. The sharp change of slope in the curves for tomato juice and Lima beans has suggested the presence of two predominant forms of thiamine, with one form being significantly more heat sensitive than the other. Secondly, since the greatest change in slope of the curves shown in Figures 2, 3, and 4 may be observed during the first 20 or 30 minutes of processing after which the curves are essentially straight, it may be postulated that naturally occurring reactive constituents of the product are depleted during the initial stages of the processing. In such instances, it might be expected that reprocessing a product which has previously undergone an extensive heat treatment would result in thiamine losses according to the first-order equation. Experiments with corn were designed to verify or disprove this theory.

Frozen corn, purchased on the open market, was homogenized in a Waring Blendor with an equal weight of distilled water, after which the slurry was processed in No. 2 cans for 80 minutes at 245°F.(118°C.). At this point the corn slurry had a pH of 6.67.

Thiamine retention studies were then made on the corn, as received, and also on the processed slurry. In each case, retentions were observed with and without the addition of phosphate buffer to stabilize the pH at approximately 6.67, the value found for corn after 80 minutes of processing. In order to raise the thiamine content to a more practical level from an analytical standpoint, 0.200 mg. of thiamine per 100 g. of corn was added to each of the four corn slurries before processing in 208 x 006 cans. The initial composition of each series, together with the observed pH and thiamine retentions after processing for various intervals at 245°F. (118°C.) is tabulated in Table 2.

Figure 6, which has been constructed from the data of Table 2, clearly demonstrates that the use of reprocessed corn is not a means of achieving less deviation from a first-order reaction rate. The accelerating influence of phosphate buffer is readily apparent from the curves of Figure 6, which substantiates the work of Farrer *(9)*, who has shown its effect in pure solutions.

In order to further observe the effect of pH on the reaction rate of thiamine, the addition of buffering agents was extended to tomatoes and Lima beans. In this instance, citrate buffer was selected as a means for obtaining a pH range of approximately two units in the neighborhood of the normal pH of the product, that is, pH 4.20 and 6.65 for tomato juice and Lima beans, respectively. Both the buffered and non-buffered products were processed at the normal pH of the product. Processing was carried out at two temperatures, 245° and 260°F.(118° and 126.5°C.) for each of the products.

In Figures 7 and 8, the percentage retentions of thiamine in Lima beans and tomato juice are plotted against the times of processing. In all cases, the higher pH values exhibited an accelerating effect upon the decompo-

TABLE 2

Comparison of thermal stability of thiamine in heated vs. reheated corn

Sample	Process time at 245°F.	pH	Retention[b] of thiamine
	minutes		%
Processed corn 2 parts plus water 1 part	0	6.65	100
(Initial thiamine content 1.47 mg./100 g.)	10	6.50	62
	20	6.35	52
	50	6.20	39
	80	6.20	29
Processed corn 2 parts plus buffer[c] 1 part	0	6.75	100
(Initial thiamine content 1.98 mg./100 g.)	10	6.75	43
	20	6.70	30
	50	6.70	12
	80	6.75	4
Frozen corn 2 parts plus water 2 parts	0	7.40	100
(Initial thiamine content 1.67 mg./100 g.)	10	7.00	70
	20	6.80	58
	50	6.40	36
	80	6.35	22
Frozen corn 2 parts plus water 1 part	0	6.75	100
plus buffer 1 part	10	6.75	42
(Initial thiamine content 1.75 mg./100 g.)	20	6.75	25
	50	6.80	7
	80	6.65	3

[b] On dry basis.
[c] 1.5 *M*. Phosphate buffer.

sition rate. The addition of citrate buffer was found to accelerate the destruction of thiamine in Lima beans, while this was not the case for tomato juice processed at 245°F.(118°C.). However, at 260°F.(126.5°C.) a slight accelerating effect was noted for the citrate buffer in tomato juice. In this connection, it is probably significant that tomato juice naturally contains an appreciable concentration of citrate.

From the data presented in this paper and in others, it may be concluded that the rate and nature of thiamine destruction in foodstuffs is governed by the combined influence of numerous factors. It also may be concluded that reliable quantitative predictions of the behavior of thiamine during thermal processing will not be justified until exhaustive studies have been made of the individual factors which up to the present have been observed only in a qualitative or semi-quantitative manner. In spite of this rather discouraging aspect, certain generalizations can be made with little or no reservation. One such generalization may be stated as follows:

The effect of temperature on the reaction rate of thiamine as compared to the destruction of microorganisms is such that the retention of thiamine during sterilization will be favored by high-temperature, short-time processing methods. Expressed in another manner, it may be said that for two thermal processes having equal lethal effect toward microorganisms, the process having the higher temperature will be more favorable to high retention of thiamine. This conclusion may be verified by reference to Figures 1 to 4, and to commercial practice that an increase of 15°F.(8°C.)

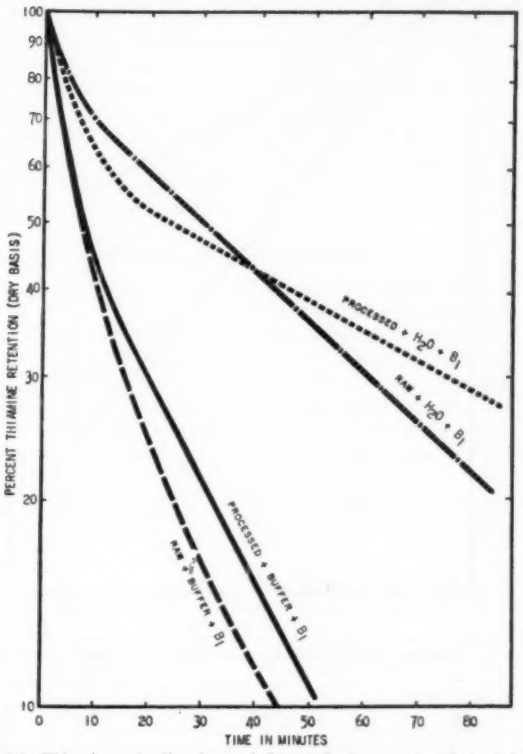

FIGURE 6. Thiamine retention in puréed corn during reprocessing at 245°F.

in processing temperature will permit a reduction in time by a factor of 0.135 without affecting the lethal characteristics of the process. Thus by comparing the thiamine retention at different temperatures for times corresponding to equivalent processes in terms of lethal effects, it will be found that in all cases the higher processing temperature is more favorable to the retention of thiamine.

Table 3 shows such a comparison at 245° and 260°F. (118° and 126.5°C.) as determined from Figures 1 to 4, inclusively.

TABLE 3

Thiamine retention at equivalent thermal processes

Process	Thiamine retention in			
	Peas	Corn	Tomato juice	Lima beans
	%	%	%	%
60 minutes at 245°F.	48	27	73	61
8 minutes at 260°F.	82	52	82	65

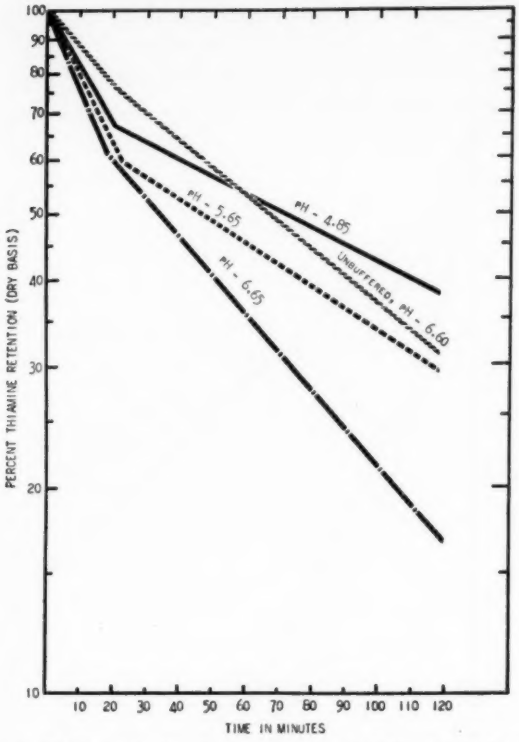

FIGURE 7. Thiamine retention during processing of buffered puréed Lima beans at 245°F.

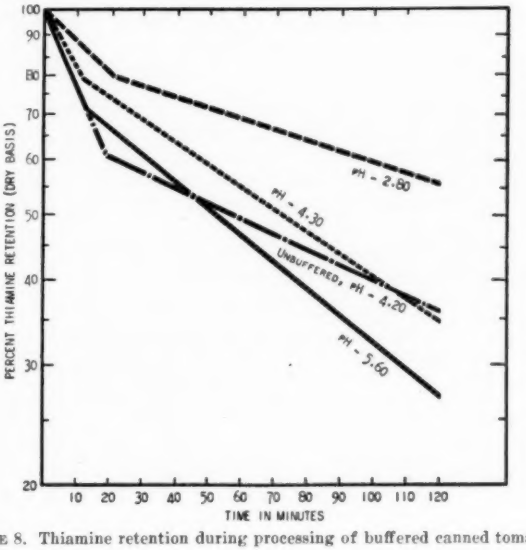

FIGURE 8. Thiamine retention during processing of buffered canned tomato juice at 245°F.

Reference has been previously made to the use of thiamine by some investigators *(5)*, as an index to other chemical changes which occur during thermal processing. From the data herein reported, it would appear that the use of thiamine retentions in this manner is open to criticism if more than qualitative significance is given to the index. In this connection, it may be pertinent that the destruction of thiamine in peas occurred in a more predictable manner as compared to the destruction in the other vegetables studied. This observation suggests the use of peas in preference to other vegetables for the evaluation of thermal processing equipment or methods.

SUMMARY AND CONCLUSIONS

A study of thiamine stability in peas, corn, Lima beans, and tomato juice during thermal processing has shown that for peas the mechanism of thiamine losses is not altered over the temperature range 220° to 270°F. (104.5° to 132.5°C.). A similar conclusion for the thiamine losses in corn, Lima beans, and tomato juice is not justified, because the reaction in these products could not be made to proceed in a first-order manner.

In all cases, the data suggest the use of high-temperature, short-time thermal processes as a means for improving the retention of thiamine in heat sterilized foods.

The use of peas in preference to other vegetables is suggested in the evaluation of new methods for effecting sterilization of foods by heat.

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AMINO ACID UTILIZATION BY SELECTED SPECIES OF *ACHROMOBACTER*[a]

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(Manuscript received May 31, 1951)

Evidence in the literature reveals that members of the genus *Achromobacter* are of much importance in the spoilage of fresh foods. Various members of the group have been incriminated in the souring of ham *(4)*, in the formation of slimy beef *(3)*, in the formation of "whiskery" beef *(4)*, in the development of mustiness of eggs *(7)*, and in the spoilage of various kinds of fish *(5)*. Many other examples could be cited but it is sufficient to say that members of the genus have been isolated from nearly all types of unprocessed food which have been examined bacteriologically.

In a study of the microbiology of shrimp taken from the Gulf of Mexico, a large proportion of the bacteria isolated, especially from shrimp undergoing spoilage, were *Achromobacters.* When an attempt was made to classify these to species according to the scheme given in Bergey's Manual *(1)* considerable difficulty was encountered. An extensive study of original isolations and of strains of as many known species as could be obtained was undertaken in an effort to improve the classification scheme. Among the characters studied as a possible tool for separation of species was the utilization of amino acids. The present report presents the results which were obtained.

Only one previous report on the amino acid utilization by members of the genus *Achromobacter* was found upon a search of the literature. During the progress of this work Sguros and Hartsell *(6)* reported on the action of *Achr. butyri* and *Achr. guttatum* on 23 amino acids in chemically defined media. They observed for these species that single amino acids could be used as both nitrogen and carbon sources, and none was essential for growth. Ammonium ion also could be used as a source of nitrogen.

METHODS AND MATERIALS

Cultures. Twenty-two species were included in the study. *Achromobacter stationis, Achr. thalassius,* and *Achr. aquamarinus* were obtained from Dr. C. E. Zobell; *Achr. lipid.s* from Dr. O. Wyss; *Achr. butyri* and *Achr. guttatum* from Dr. S. E. Hartsell; *Achr. perolens, Achr. globiforme, Achr. hartlebii,* and *Achr. lipolyticum* from the American Type Culture Collection; *Achr. amylovorum, Achr. liquefaciens, Achr. alcaliaromaticum, Achr. delicatulum, Achr. superficiale, Achr. eurydice,* and strain C 96 were isolates from spoiled shrimp; strains M 8, M 14, M 23, N 43, and N 45 were isolates from chitin enrichment cultures. These species designations include several which were included in the 5th Edition of Bergey's Manual but are not recognized in the 6th. Four of the species listed in the 6th Edition could not be obtained.

Materials. Pyrex glassware, aluminum caps, and glass distilled water were used throughout the work. All glassware was cleaned by soaking overnight in potassium

[a] This investigation was supported by a research grant from the National Institutes of Health, U. S. Public Health Service.

dichromate cleaning solution, rinsed thoroughly in tap water, then in distilled water, and allowed to air dry.

Media. The medium employed was adapted from that described by Dunn *(2)*. It is a chemically defined medium containing amino acids, minerals, and glucose of commercial chemically pure grades. For its exact composition see Table 1.

TABLE 1

Basal medium used for determination of amino acid requirements

Constituent	Amount	Constituent	Amount
DL-Alanine	100 mg.	DL-Serine	100 mg.
L-Arginine	100 mg.	DL-Threonine	100 mg.
L-Aspartic acid	400 mg.	DL-Tryptophan	150 mg.
L-Cystine	100 mg.	L-Tyrosine	100 mg.
L-Glutamic acid	1 g.	DL-Valine	100 mg.
L-Glycine	100 mg.	Glucose	10 g.
L-Histidine	100 mg.	NH_4Cl	6 g.
L-Hydroxyproline	100 mg.	KH_2PO_4	0.5 g.
DL-Isoleucine	100 mg.	K_2HPO_4	0.5 g.
L-Leucine	100 mg.	$MgSO_4 \cdot 7H_2O$	0.25 g.
DL-Lysine	100 mg.	$MnSO_4 \cdot 4H_2O$	0.1 g.
DL-Methionine	100 mg.	NaCl	0.1 g.
DL-Phenylalanine	100 mg.	$FeSO_4 \cdot 7H_2O$	0.01 g.
L-Proline	100 mg.	Distilled water	1000 ml.

The procedures followed for the determination of the availability of the various amino acids for growth through the use of this medium included the omission of individual amino acids in order to see if any one was essential for growth, the omission of a group of related amino acids in order to determine if there was a requirement for one member of the group which could be supplied by a related member, and by determinations of the availability of each amino acid as a source of carbon in the presence of ammonium chloride, and as a source of nitrogen in the presence of glucose.

The groups of related amino acids which were tested are as follows: 1, alanine and glycine; 2, valine, leucine, and isoleucine; 3, serine and threonine; 4, cystine and methionine; 5, aspartic acid and glutamic acid; 6, proline and hydroxyproline; 7, phenylalanine, tyrosine, and tryptophan; and 8, arginine, lysine, and histidine. The test media were pipetted into sterile tubes in 5-ml. amounts and were autoclaved at 10 p.s.i.g. steam pressure for 15 minutes. The pH of all media was 7.0 to 7.2.

Preparation of inoculum. Cultures were grown in 1% Albimi peptone C broth for 20 to 24 hours at room temperature. The cells were harvested by centrifugation, washed three times with physiological salt solution, and resuspended. Plate counts on suspensions prepared in this manner showed from 5,000 to 100,000 viable cells per ml.

Tubes of test media were inoculated with 0.1 ml. of a cell suspension prepared as described above and a total of four serial transfers were made at 24-hour intervals with a loop needle. Growth response was detected by turbidimetric measurement.

RESULTS AND DISCUSSION

Good growth was obtained in a glucose-mineral salts-ammonium chloride medium, although all strains grew faster in the complete medium. This can possibly be explained on the assumption that the amino acids are being assimilated as such, with less effort being required for growth than would be the case if the synthesis of cell constituents took place by utilization of inorganic nitrogen and glucose as the sole source of nitrogen and carbon.

No one of the amino acids was required for growth of any of the strains studied. Individual amino acids permitted satisfactory growth in

the presence of glucose, from which it appears that single amino acids can be used as a source of nitrogen for the synthesis of all nitrogenous constituents of the cell. According to modern concepts of amino acid synthesis, it is plausible that the synthesis of amino acids by *Achromobacter* strains takes place by the utilization of ammonia and alpha keto acid(s). If this is the case, then one can interpret the utilization of individual amino acids as a means whereby ammonia is obtained for the synthesis of nitrogenous cell constituents.

All the species and strains tested were able to use each of the amino acids as a source of carbon as well as nitrogen with varying degrees of luxuriance of growth in serial transplants with a few exceptions: *Achr. superficiale* was unable to obtain carbon from alanine and aspartic acid; *Achr. aquamarinus* and *Achr. lipolyticum* were unable to use valine; and *Achr. lipidis* and *Achr. alcaliaromaticum* were unable to use arginine.

All strains were able to obtain their sulfur from an inorganic source. None required a preformed growth factor.

This report indicates that the genus *Achromobacter* is not exacting in requirements for growth, a fact which may well account for its wide distribution in nature. It is clear that it is not possible to separate species on the basis of differences in amino acid utilization.

SUMMARY

It has been established for 22 different members of the genus *Achromobacter* that no one of 19 amino acids tested was required for growth. All species were able to use each amino acid and ammonium chloride as a source of nitrogen. There were a few species which could not obtain carbon for growth from all of the amino acids.

Preformed growth factors were not required. Sulfur requirements could be supplied by inorganic sulfur.

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DIKETOGULONIC ACID, DEHYDROASCORBIC ACID, AND ASCORBIC ACID CONTENT OF FOUR FRUITS[a]

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(Manuscript received June 20, 1951)

In 1948 we published data *(1)* on the dehydroascorbic acid and ascorbic acid contents of cantaloupes. During the time that this earlier work was in progress, evidence was accumulating which indicated that the dinitrophenylhydrazine procedure of Roe and Oesterling *(2)* which we used also measured diketogulonic acid. In 1948 Roe, Mills, Oesterling, and Damron *(3)* published procedures which made possible the use of the diphenylhydrazine method for the identification of ascorbic acid (AA), dehydroascorbic acid (DHAA), and diketogulonic acid (DKGA) in the presence of each other. Information on the DHAA content of foods is meager and there is little or no information on their DKGA content. Therefore, we felt it would be of interest to extend our earlier study by making further analyses on cantaloupes for these three constituents. It was thought that the "total ascorbic acid" previously reported might include an appreciable amount of DKGA which appears to be biologically inactive. The three compounds were also determined in grapefruit, pineapple, and strawberries.

PROCEDURE

The cantaloupes were of known varieties obtained through the Department of Horticulture of Purdue University directly from the field. Analyses were carried out immediately and after 24 hours of storage in the refrigerator. Halves of melons cut for immediate analysis were stored both wrapped and unwrapped. Samples for analysis were obtained from each melon by cutting crosswise with a plastic knife and taking 25 g. of the edible portion.

The grapefruit, pineapple, and strawberries were purchased on the open market. Information on the exact variety was not obtainable. The grapefruit was of a white seedless variety shipped from Texas in January and February. The pineapples were purchased late in May and early in June. The strawberries were Louisiana berries shipped in April, and Kentucky and Indiana berries shipped in June. Each quart of strawberries from which samples were taken for analysis was bought from a different store.

As with the cantaloupe, two analyses were made on the grapefruit, one immediately after cutting and the other after 24 hours of storage in the refrigerator. Grapefruit and pineapple samples were obtained in the same way as the cantaloupe samples. A few strawberries were selected from a quart box and mixed to prepare a sample for analysis. The dinitrophenylhydrazine method of Roe et al. *(3)* was employed for all analyses.

RESULTS

Table 1 gives the results of the analyses on four known varieties of cantaloupe and on grapefruit, pineapple, and strawberries, the exact vari-

[a] This work was aided by a grant from the Purdue Research Foundation.

[b] Based on material constituting part of a doctoral thesis submitted to the graduate school of Purdue University.

TABLE 1

Diketogulonic acid (DKGA), dehydroascorbic acid (DHAA), and ascorbic acid (AA) contents of various fruits

Fruit	Variety	Time	No. of samples	DKGA	DHAA	AA
				mg./100 g.	*mg./100 g.*	*mg./100 g.*
Cantaloupe	P44	July and Sept.	10	2.46 (0.78-4.23)[a]	8.17 (5.72-14.47)	33.28 (22.25-49.56)
Cantaloupe	Pride of Wisconsin	August	6	2.60 (1.98-3.50)	7.74 (3.47-11.59)	41.75 (32.06-50.40)
Cantaloupe	MR54	August	2	2.03 (1.45-2.60)	7.23 (7.09-7.37)	25.37 (22.43-28.32)
Cantaloupe	Lafayette Austin	September	6	2.04 (1.76-2.83)	9.69 (3.59-17.94)	22.61 (7.28-37.84)
Grapefruit	A Texas seedless	Jan. and Feb.	12	1.08 (0.70-2.39)	0.96 (0.68-1.60)	41.80 (33.90-48.44)
Pineapple	Not known	May and June	6	0.93 (0.83-1.08)	2.05 (1.63-3.08)	19.35 (14.78-26.84)
Strawberries	Not known	April	4	2.70 (1.49-5.11)	6.60 (4.17-8.93)	63.54 (65.51-77.93)
Strawberries	Not known	June	3	1.66 (1.13-1.91)	2.14 (2.06-2.21)	79.95 (69.59-87.09)

[a] Figures in parentheses show range; single value is average of all analyses.

eties of which were not known. Both AA and DHAA as well as DKGA were found in all of the fruits. However, the relative proportions varied considerably. The percent of DKGA was the highest in the cantaloupe and in April strawberries and the lowest in the grapefruit, pineapple, and June strawberries. This was also true of the DHAA. AA amounted to about 95% of the total of the three compounds in the grapefruit and June strawberries; 70 to 80% in the cantaloupe; and about 85% in the April strawberries and the pineapple. There were not only the expected varietal differences among the cantaloupes but the range of values for individual samples of a given variety were often as great as that for the other fruits which were purchased on the open market.

In Table 2 the values for AA and DKGA are given for cantaloupe and grapefruit analyzed immediately after cutting and following storage in the refrigerator for 24 hours. Little change occurred in the stored grapefruit, but there was an appreciable increase in the DKGA content of the cantaloupe during storage. This was accompanied by a decrease in the DHAA content. Little difference was found between the wrapped and unwrapped melons. This is in agreement with our earlier study on cantaloupes.

Since DHAA, which appears to have a biological availability close to that of AA, may be present in considerable quantity in certain fruits, more information is needed for the revision of some of the published values for ascorbic acid content of foods since most of the older figures appearing in tables are based on determination of AA only.

TABLE 2

Distribution of diketogulonic acid (DKGA), dehydroascorbic acid (DHAA), and ascorbic acid (AA) of freshly cut and stored halves of cantaloupe and grapefruit[4]

	Freshly cut			Stored in refrigerator					
				Wrapped			Unwrapped		
	DKGA	DHAA	AA	DKGA	DHAA	AA	DKGA	DHAA	AA
Cantaloupe									
Avg. of 18 samples, mg./100 g.	2.17	8.36	26.93	5.82	6.63	28.33	5.91	8.26	33.06
Percent of total[4]	7.9	19.6	72.5	15.0	15.6	69.4	13.4	17.1	69.5
Grapefruit									
Avg. of 7 samples, mg./100 g.	0.80	0.88	38.56	0.97	1.00	39.28	1.09	1.03	39.95
Percent of total[4]	2.0	2.2	95.7	2.4	2.4	95.2	2.6	2.5	94.9

[4] These values express the three compounds as percentages of the sum total of the three compounds.

SUMMARY

Four varieties of cantaloupe, and samples of grapefruit, pineapple, and strawberries have been analyzed for diketogulonic acid (DKGA), dehydroascorbic acid (DHAA), and ascorbic acid (AA). Cantaloupes and grapefruit were analyzed immediately after cutting and following storage of the cut halves in the refrigerator for 24 hours.

All three compounds were found to be present in the four fruits, but in varying proportions.

The grapefruit showed little change as result of storage, but there was an increase in the diketogulonic acid content of the stored cantaloupes accompanied by a decrease in dehydroascorbic acid. Little difference was observed between the wrapped and unwrapped cantaloupes.

There is a need for re-evaluation of the ascorbic acid content of fruits and vegetables, based on late methods of analysis, since most of our published figures give a measure of ascorbic acid only.

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EFFECT OF ALLYLISOTHIOCYANATE AND RELATED SUBSTANCES ON THE THERMAL RESISTANCE OF *ASPERGILLUS NIGER, SACCHAROMYCES ELLIPSOIDEUS,* AND *BACILLUS THERMOACIDURANS*[a]

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In a previous report we reviewed the use of mustard seed and allylisothiocyanate (mustard oil) as preservatives for fruit juices *(3)*, and preliminary data on the preservation of fresh cider and grape juice by these substances was reported. It was indicated that allylisothiocyanate in concentrations of approximately 20 p.p.m., exerted a favorable preservative action on fresh cider.

The present investigation was carried out to ascertain the effect of allylisothiocyanate, oil of garlic, and oil of onion on the thermal resistance of *Aspergillus niger, Saccharomyces ellipsoideus,* and *Bacillus thermoacidurans.* These organisms were examples of spoilage microorganisms that might be encountered in acid foods.

EXPERIMENTAL PROCEDURE

Microorganisms

The representative test organisms selected were *Aspergillus niger* and *Saccharomyces ellipsoideus,* taken from stock cultures in this laboratory, and *Bacillus thermoacidurans,* strain number 8038 *(Bacillus coagulans)* from the American Type Culture Collection.

Substrates

Citric acid-dibasic sodium phosphate buffer solutions for pH 4.0 and 7.0 were prepared according to McIlvaine *(4)*. Freshly pressed apple juice was filtered and held in the frozen state in glass containers prior to use. The juice employed had a pH value of 3.9 and a soluble solids content of 13.8%. Pasteurized Concord grape juice was prepared in the laboratory. It had a pH value of 3.8 and a soluble solids content of 16.5%. A given lot of washed, trimmed tomatoes cut into quarters, were placed in a steam-jacketed kettle and heated to boiling. Then the hot pulp was passed through a cyclone finisher and the juice was then filled at 87.7°C.(190°F.) into No. 10 (603 x 700) unlined cans which were processed in boiling water.

Preparation of Spore and Yeast Cell Suspensions

The *Aspergillus niger* spore crops were grown on slants of dextrose agar (Difco) and apple juice agar (prepared by adding 2% agar to apple juice) for 12 days at room temperature [21° to 24°C.(70° to 75°F.)]. The spores were washed from the agar slants with sterile physiological saline and collected in a sterile flask. The spore suspension was then filtered through a sterile cheese cloth cotton filter pad and the filtrate placed in 50-ml. centrifuge tubes and centrifuged for 15 minutes at 3,000 r.p.m. to concentrate the spores. The spores were washed from the centrifuge tube with 5 ml. of saline and placed in a sterile flask containing glass beads. The spore concentration was

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determined by direct count with a haemocytometer. Immediately before use the spore suspension was shaken for 15 minutes to aid in breaking up clumps.

Saccharomyces ellipsoideus was grown on wort agar slants for four days at room temperature [21° to 24°C.(70° to 75°F.)]. Three transplants were made at four-day intervals. The yeast cells were washed from the agar slants of the third transfer and were suspended in buffer solution (pH 4.0) or grape juice. The cell concentration was determined by plating on wort agar.

Bacillus thermoacidurans crops and suspensions were prepared as described by Anderson, Esselen, and Fellers *(1)*. This procedure was proposed by Stern, Hegarty, and Williams *(5)* with modifications as indicated.

Method for Determining Thermal Resistance

Aspergillus niger. The multiple tube method of Esty and Williams *(2)* was followed in determining the thermal death time characteristics of the spores of *Aspergillus niger*. The apparatus used consisted of a 3½-gallon electrically heated oil bath controlled to within ± 0.3°C.(0.5°F.) with a DeKhotinsky thermoregulator. Thermal death time tubes were filled with 2 ml. of the substrate containing 10,000 spores per ml. by means of a sterile 20-ml. syringe graduated in 2-ml. increments. The tubes were sealed in an oxygen flame, and sets of five tubes were placed in baskets, made of copper screening, for the heat treatment. Wire handles were soldered to the baskets so that the tubes could be completely immersed in the heating medium.

Five tubes containing the spore suspension to be tested were used for each time interval at each temperature. At least 5 time intervals were used at each temperature. For *Aspergillus niger* the four temperatures used were 54.4°C.(130°F.), 60°C.(140°F.), 65.6°C.(150°F.), and 71.1°C.(160°F.). The tubes were immersed in the oil bath for the specified time, and then the basket and tubes were removed from the oil bath and cooled in ice water. The tubes were freed of oil, flamed, and opened by means of a device similar to that described by Williams *(6)*. The tubes were subcultured in dextrose broth (pH 6.5). They were incubated at 25°C.(77°F.) for 15 days and then recorded as positive or negative depending upon the presence or absence of growth.

The results of these determinations were then plotted as survival and destruction points against time and temperature on semilogarithmic paper. A line drawn so as to be above all the survival points but below as many destruction points as possible was designated as the thermal death time curve and reported as F_{160} and z values where F_{160} is the destruction time at 160°F.(71.1°C.); and z is the slope of the curve.

Saccharomyces ellipsoideus. With this organism, thermal destruction rates were determined at 38.9°C.(120°F.), 54.4°C.(130°F.), and 60°C.(140°F.). Three thermal death time tubes containing 2 ml. of substrate (400,000 cells per ml.) were heated for each time period as already described. One-ml. portions of the heated substrates were diluted and plated on wort agar plates in duplicate to determine the number of surviving organisms. The plates were incubated for 5 days at 25°C.(77°F.). Destruction rate curves were plotted on semilog paper, and the "D" values determined. The "D" value represents the time required for 90% destruction of the organisms. Straight line destruction rate curves were obtained. The "D" values were plotted against temperature on semilog paper in order to derive the z values.

Bacillus thermoacidurans. The thermal death rate for *Bacillus thermoacidurans* was determined as already described by using a concentration of 300,000 spores per ml. in the substrate. Destruction rate curves were obtained at temperatures of 87.7°C.(190°F.), 93.3°C.(200°F.), and 98.9°C.(210°F.). Dilutions of the heated substrates were plated out on the proteose peptone acid agar media of Stern, Hegarty, and Williams *(5)* as described by Anderson, Esselen, and Fellers *(1)*. The plates were incubated for 72 hours at 37°C.(98.6°F.).

The effects of media on which the spores were grown, of the pH of buffer solution, of added allylisothiocyanate (mustard oil) in buffer at pH 4.0, 10% dextrose solution, of 0.75% tartaric acid solution, and the influence of apple juice on the heat resistance of the spores of *Aspergillus niger* were determined and are shown in Table 1. The effects of 10 p.p.m. allylisothiocyanate on the thermal death rate of *Saccharomyces ellipsoideus* in buffer solution at pH 4.0 and in grape juice are presented in Table 2. Data on the effect of added allylisothiocyanate, oil of garlic, and oil of onion on the

TABLE 1

Effect of allylisothiocyanate on the thermal resistance of *Aspergillus niger* spores (10,000 spores per ml. of substrate)

Substrate	F_{180} value	z value
Buffer, pH 4.0 (spores grown on apple juice agar)	1.2	28.5
Buffer, pH 4.0 (spores grown on dextrose agar)	1.2	33.0
Buffer, pH 7.0	1.8	30.5
Buffer, pH 4.0 plus 10 p.p.m. allylisothiocyanate	0.2	20.8
Buffer, pH 4.0 plus 25 p.p.m. allylisothiocyanate	0.1	24.0
10% dextrose solution	0.8	28.0
10% dextrose solution plus 10 p.p.m. allylisothiocyanate	0.3	31.7
0.75% tartaric acid solution	0.6	31.0
0.75% tartaric acid solution plus 10 p.p.m. allylisothiocyanate	0.1	24.0
Apple juice, pH 3.9	0.7	25.4
Apple juice, pH 3.9, plus 10 p.p.m. allylisothiocyanate	0.1	22.0

TABLE 2

Effect of allylisothiocyanate on the thermal resistance of *Saccharomyces ellipsoideus* (400,000 cells per ml. of substrate)

Substrate	D value[c]			F_{180} value[d]	z value
	120°F.	130°F.	140°F.		
Buffer, pH 4.0	6.0	3.5	2.2	3.2	46.0
Buffer, pH 4.0, plus 10 p.p.m. allylisothiocyanate	3.0	2.0	1.05	1.6	44.0
Grape juice, pH 3.8	5.9	3.4	2.0	2.3	42.5
Grape juice, pH 3.8, plus 10 p.p.m. allylisothiocyanate	2.9	1.6	1.0	1.4	43.2

[c] Time in minutes for 90% destruction.
[d] Time in minutes for 99.99% destruction at 160°F. from extension of thermal death time curve.

thermal death rate of *Bacillus thermoacidurans* in buffer solution at pH 4.0 and in tomato juice are summarized in Table 3.

In these heat resistance tests a previously determined correction of 2.1 minutes was allowed for the lag period for the contents of the thermal death time tubes to reach the temperature of the heating bath.

DISCUSSION OF RESULTS

The addition of 10 p.p.m. of allylisothiocyanate to the various substrates tested was very effective in lowering the thermal resistance of the spores of *Aspergillus niger* (Table 1). To check the effectiveness of 10 p.p.m. allylisothiocyanate in this respect, a small inoculated pack of apple juice was made. Groups of 12 pint jars each of sterilized apple juice and sterilized apple juice plus 10 p.p.m. allylisothiocyanate, respectively, were inoculated with 15,000,000 *Aspergillus niger* spores per jar. Groups of jars were then immediately pasteurized in a water bath at 71.1°C.(160°F.) for 1, 3, 5, 7, 9, 11, 13, and 15 minutes, respectively. The jars were air cooled, and portions of each were subcultured in malt extract broth. Viable mold spores were recovered from the control jars pasteurized up to and including 13 minutes but were not recovered from jars containing allylisothiocyanate pasteurized more than 5 minutes.

TABLE 3

Effect of allylisothiocyanate, oil of garlic, and oil of onion on the thermal resistance of *Bacillus thermoacidurans* (300,000 spores per ml. of substrate)

Substrate	D value[a]			F_{212}[b] value	z value
	190°F.	200°F.	210°F.		
Buffer, pH 4.0	59.0	22.0	9.5	32.0	25.5
Buffer, pH 4.0, plus 20 p.p.m. allylisothiocyanate	42.4	16.0	6.0	20.0	23.7
Buffer, pH 4.0, plus 20 p.p.m. oil of garlic	53.0	21.0	7.5	25.6	23.4
Buffer, pH 4.0, plus 20 p.p.m. oil of onion	56.0	21.4	8.6	28.0	24.5
Tomato juice, pH 4.3	72.5	23.0	8.5	30.4	22.4
Tomato juice, pH 4.3, plus 20 p.p.m. allylisothiocyanate	65.0	20.8	7.2	23.2	21.1
Tomato juice, pH 4.3, plus 50 p.p.m. allylisothiocyanate	55.0	17.2	6.0	19.0	20.7

[a] Time in minutes for 90% destruction.
[b] Time in minutes for 99.99% destruction at 212°F. from extension of thermal death time curve.

The addition of 10 p.p.m. allylisothiocyanate was also effective in reducing the thermal resistance of *Saccharomyces ellipsoideus* (Table 2). A series of pint jars of sterilized grape juice, with and without added allylisothiocyanate, was prepared as in the apple juice test with *Aspergillus niger*. Each jar was inoculated with 16,000,000 yeast cells. After pasteurization at 71.1°C.(160°F.), aliquots of each jar were plated out on wort agar. The results indicated that pasteurization for 13 minutes was required to destroy the yeasts in the control juice as compared with 4 minutes for the juice containing 10 p.p.m. allylisothiocyanate.

Allylisothiocyanate had less effect on the heat resistance of *Bacillus thermoacidurans* than it did on *Aspergillus niger* and *Saccharomyces ellipsoideus* (Table 3). Oil of garlic and oil of onion showed some influence, but less than that of allylisothiocyanate, in lowering the heat resistance of *Bacillus thermoacidurans*. It should be pointed out that the concentration of allylisothiocyanate in these tests is far in excess of that which could be tolerated in a food product.

The results of this investigation indicate that the strains of *Aspergillus niger* and *Saccharomyces ellipsoideus* tested are very sensitive to the presence of 10 p.p.m. allylisothiocyanate, insofar as their thermal resistance is concerned, whereas *Bacillus thermoacidurans* is much less sensitive to this compound. Although these results may not be directly applicable to the preservation of acid foods, they suggest that the presence of allylisothiocyanate (mustard oil) as an ingredient in acid foods may enhance the ease with which molds and yeasts may be controlled.

CONCLUSIONS

The presence of 10 p.p.m. of allylisothiocyanate (mustard oil) in buffer solutions and in apple and grape juices caused a marked reduction in the

thermal resistance of *Aspergillus niger* and *Saccharomyces ellipsoideus*, but *Bacillus thermoacidurans* was much less sensitive to this compound. Oil of garlic and oil of onion had less effect on the thermal resistance of *Bacillus thermoacidurans* than did allylisothiocyanate.

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SOME ASPECTS OF MICROBIOLOGY APPLIED TO COMMERCIAL APPLE JUICE PRODUCTION. II. MICROBIOLOGICAL CONTROL OF PROCESSING

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Microbiological control of apple juice processing consists of routine assessments of the numbers of microorganisms present in the juice at all stages of production, a determination of the biological cleanliness of the processing equipment, and as a check on the efficiency of the plant. It is not intended, in this paper, either to suggest a rigid scheme of microbiological control suitable for general application, or to describe a system that has been adopted for the plant where this investigation was carried out. Our main object is to illustrate that microbiological methods can prove of extreme value in serving the functions mentioned above and to ensure that risk of spoilage from the organisms invariably present in the juice is at all times reduced to a minimum.

This investigation was carried out in a factory where the Boehi system of storing juice in bulk in pressure tanks prior to bottling had been installed. The Boehi process together with the necessary ancillary plant has been described in some detail by Charley and Harrison *(2)* and a typical layout is illustrated diagrammatically in Figure 1.

Microbiological control is desirable at each stage for the purpose of ascertaining that there has been no increase in the number of microorganisms in the juice.

PROCESSING CONTROL

If microbiological control is to yield results of significance, samples must be taken at stipulated times after each processing stage has been completed, and cleanliness of plant and equipment must be adequate and rigidly enforced. Correct interpretation of the data obtained will prove of value only if the methods of sampling are studied with due care and intelligence. If production of apple juice is carried out on a large scale and a sufficient number of routine tests can be carried out, the accuracy and significance of the recorded data can undoubtedly be raised by statistical treatment. In fact, compilation and utilization of statistical control charts could prove of inestimable value. The data presented in this paper has not been submitted to statistical treatment, but the possibilities of application of such methods will be readily apparent.

Fruit pressing stage. In the first paper of this series the authors *(4)* showed that the population densities of the apple microflora exhibit considerable variation. It may be anticipated that a relationship will exist between the number of microorganims present on the fruit and that in the freshly expressed juice. Consequently, the latter should also possess an initial microbial population that will vary according to the numbers present on the fruit, or in the pulp immediately after milling. This relationship is, however, subject to certain reservations and will depend upon the time required

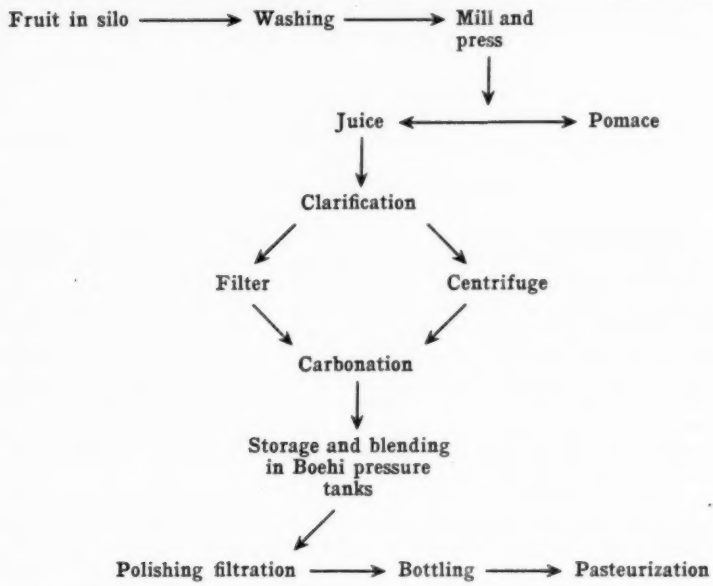

FIGURE 1. Flow sheet for the production of apple juice.

for pressing, the sanitary condition of the press and press cloths, the temperature of the expressed juice, and the interval that is allowed to elapse before the juice is sampled after pressing. With the exception of the sanitary condition of the pressing equipment, the remaining factors are amenable to control, and must, therefore, be maintained constant.

Under ideal conditions the total number of microorganisms in freshly expressed juice will always be less than the total number on the fruit. This is readily illustrated by a laboratory pressing experiment. A batch of apples, visually sound, were weighed and halved aseptically. The total yeasts and molds were determined in one half of each apple by the pulping technique previously described by the authors *(4)* and the average number of organisms calculated per ounce (28.35 g.) of pulp. The remaining halves of the apples were pulped and pressed in the laboratory. The yield of juice from 1 oz. (28.35 g.) of pulp was found to be 20 ml. Counts were made of the yeasts and molds present in 1 ml. of the freshly expressed juice. Compared with the calculated figures, the numbers actually present were much lower (Table 1).

TABLE 1

Relation between microbiological population of apples and that of the freshly expressed juice

Organisms	Microorganisms per oz. (28.35 g.) apple	Microorganisms per ml. expressed juice	
		Calculated	Found
Yeasts	8900	445	324
Molds	640	32	18

It will be apparent that to a certain extent the milled pulp behaves as a filter in trapping a large proportion of the microorganisms which remain behind in the pomace after pressing. This can easily be demonstrated by suspending the fresh pomace in sterile water, intimately mixing it, and withdrawing samples for plate counts.

Unfortunately, it is not possible to maintain this reduction in microbial population for long on a factory plant. If the juice is left for an indefinite period before further treatment, the organisms commence to reproduce very rapidly. Apart from this, the press cloths also contribute to the increasing population of the juice. It is not practical to sterilize the cloths between each pressing, but enumeration of the yeast population at the end of the day has shown counts ranging from 1,000 to 8,000 organisms per sq. inch of cloth. Assuming that from a total surface area of 69,000 sq. inches of press cloth in one "cheese" a yield of 170 gallons of juice is obtained, then this source alone can contribute from 100 to 800 organisms per ml. of juice.

In the previous paper we demonstrated that it is possible to establish arbitrary limits for the microfloral population of apples in order to differentiate between fruit that is sound and that which would be undesirable for processing. Obviously, visual examination of the fruit provides a more rapid means of assessing its condition than does a series of microbiological counts. Moreover, it would not be practical to take frequent samples of apples from the silo for individual counts on each apple. Although such methods will provide a useful check on the efficiency of the fruit grading department, this may also be assessed indirectly in a more practical manner, to which we will refer later.

It is customary to wash thoroughly all fruit taken from the silo immediately before pressing and by this means effectively reduce the number of surface microorganisms present on sound fruit. Some typical data compiled from three different washing experiments are recorded in Table 2, showing the reduction in the total number of surface organisms per apple, before and after washing in running water. As a rule, no direct correlation can be established between the numbers removed and those originally present since the latter vary in their tenacity to adhere to the cuticle.

TABLE 2

Removal of surface organisms from apples by washing

Treatment	Average number of surface organisms per apple		
Before washing	140,000	112,000	7,000
After washing	91	108	196

The numbers of organisms still remaining on washed unsound fruit are far in excess of these figures, and for routine control, sampling of the fruit after washing for microbiological counts will still provide a reliable indication of the condition of the fruit used for processing. The most suitable stage at which to sample is immediately after the pulp has been milled. The mills are run intermittently and only when each new "cheese" is being built. Samples should be taken just before sufficient fruit has been ground to complete a "cheese" so that any yeasts that may have accumulated in the mill from the previous operation may be swept out by the fresh batch of pulp. Samples will then be more representative of the microbiological condition of the fruit that has been milled. The assays should be expressed as number of microorganisms per g. of pulp. Data obtained over a pressing season and compared with direct counts on the unwashed fruit as shown in our previous paper, will provide the plant microbiologist with a useful guide to the condition of the fruit that has been processed.

Clarification and fining. Besides removal of colloids, thereby facilitating filtration, fining is also of value for effecting a reduction in the number of microorganisms present in the juice. The number removed is influenced by temperature and by the method of clarification employed. The gelatin-tannin treatment is more preferable for this purpose than enzyme clarification, since the higher temperature required for the latter process is favorable for multiplication of the juice microflora. Even when time and temperature conditions are rigidly fixed, the efficiency of the fining treatment varies from one batch of juice to another, depending on the nature and amount of the colloids

present. Within limits, the percentage reduction in the yeast count may be relied upon to provide a rough quantitative indication of the efficiency of the fining process. The yeast and mold counts in 34 different batches of juice before and after fining are recorded in Table 3. The intervals between sampling were approximately the same for each batch.

TABLE 3

Effect of fining in reducing the number of microorganisms in apple juice

Before fining		After fining			Before fining		After fining		
Yeasts	Molds	Yeasts	Molds	Yeasts removed	Yeasts	Molds	Yeasts	Molds	Yeasts removed
				%					%
3360	1	1200	....	64.3	3200	2	250	1	93.6
840	3	310	7	63.1	9150	11	330	1	96.4
1200	8	200	2	83.4	8130	4	450	5	94.5
2400	70	280	3	88.3	2150	1	1820	3	15.5
3700	120	750	7	79.7	3400	3	730	1	78.5
7200	20	1210	12	83.2	.....	....	647	1	.....
5600	80	3210	9	42.7	4130	4	1130	3	72.6
1100	25	290	8	73.6	7180	6	2100	5	70.7
950	80	520	11	45.3	2120	2	1040	3	50.9
2150	16	160	20	92.6	3130	1	1400	2	55.3
1280	37	410	1	68.0	3470	2	1010	1	70.9
2816	3	720	1	74.4	4610	3	2100	2	56.4
1423	7	491	3	65.5	2030	2	970	1	52.2
2100	2	210	1	90.0	2100	6	1200	3	42.8
1210	9	485	2	59.9	2740	3	900	1	67.2
3720	13	1100	2	70.4	3150	4	860	2	72.7
2155	9	920	1	57.3	2210	2	750	1	66.1

Counts on the clarified juice were made on samples taken at least 12 inches above the clarification sludge at the bottom of the vat. The yeast and mold counts are not absolute values, but relative only, being expressed in terms of the lowest mold count encountered in any of these samples. This illustrates more clearly the relation between yeast and mold counts per ml. of juice. The average reduction in yeast count achieved by clarification was 62.4% but varied per batch from a minimum of 15.5% to a maximum reduction of 96.4%. From observations carried out at the time of processing it may be stated that a good relationship was evident between the clarity of the juice after fining and the reduction in yeast count. On the contrary, change in the mold count is of no value as a criterion in assessing clarification efficiency. Morphologically, molds are far more complex than yeasts, and as we have stated in the previous paper (4), a statement that a juice contains a certain number of molds per ml. only holds good under certain reservations.

Filtration and centrifuging. It is interesting to note from Table 3 the preponderance of yeasts to molds in the unclarified juice. This still persists, to a reduced extent, after clarification.

Table 4, which is a continuation of Table 3, shows the further reduction in yeast counts when the clarified juice is either centrifuged or filtered. Each batch of clarified juice was divided so that both treatments might be compared. The fairly coarse grade of filter sheet that was employed provided results comparable with those obtained by centrifuging as will be observed from examination of the yeast counts. Either treatment reduced the yeast count on the average to 3-4% of that in the unclarified juice.

The efficiency of the filter in reducing the yeast count of the juice depends upon the microbiological cleanliness of the former, the number of organisms in the juice to be filtered, and the grade of filter sheet employed. By using a filter that has been sterilized with steam after the filter sheets have been inserted the two latter principles may be clearly demonstrated. Yeast counts were made on a batch of juice immediately

TABLE 4

Comparison of efficiency of filter and centrifuge for further reduction of yeast count in juice [a]

Relative number of yeasts/ml. juice			
After filtration	After centrifuge	After filtration	After centrifuge
P	0.5	1.0	1.3
7.6	25.0	0.6	0.3
3.4	15.0	0.5	P
3.2	2.2	2.5	P
0.7	1.2	1.2	P
0.7	0.9	....	P
0.9	0.9	1.5	1.2
10.9	0.5	0.5	0.7
10.2	P	2.1	3.4
3.7	2.6	2.0	2.4
5.7	4.8	1.2	0.9
4.3	3.5	0.7	1.0
5.5	P	1.9	1.9
4.3	1.7	1.8	2.0
6.8	4.2	P	1.2
2.2	1.4	P	1.2
4.4	1.7	P	1.7

[a] The letter P indicates that a filter or centrifuge was not used.

before and after filtration. The filter was then stripped, fresh sheets of the same grade inserted, the whole assembly again sterilized with steam, and the juice given a second filtration. This was repeated a third time. Thus, juice containing diminishing numbers of yeasts was filtered under ideal conditions without risk of additional contributions to the yeast flora from a contaminated filter. This experiment was carried out on four different batches of juice, using different grades of filter sheets for each batch. From Table 5 it is apparent that by selection of a suitable grade of filter sheet according to the yeast population of the juice, and provided that contamination from the filter is excluded the yeast content of the juice can be reduced to a negligible amount.

TABLE 5

Influence of initial yeast count and grade of filter sheet on number of organisms removed

Treatment	Filter sheet grade			
	No. 1	No. 3	No. 5	No. 7
Before filtration	3270	1147	3920	82
After first filtration	34	256	14	3
After second filtration	26	323	6	0
After third filtration	2	1	0	0

Changes during storage. Representative sampling of juice for microbiological counts during storage in Bochi pressure tanks is a matter of some difficulty since it is not practical to agitate the contents. Consequently, there is a tendency for some sedimentation of microorganisms to occur and juice withdrawn from the sampling cock often shows an apparent increase in count. Care must be taken to remove a volume of juice sufficiently large to thoroughly flush the sample cock which usually forms a pocket where yeasts and mold tend to congregate. Only by care and in the light of practical experience can any reliable interpretation be placed on the examination of counts on juice in storage. Since so much depends on local conditions, presentation here of any of our recorded data would prove of very little value.

PLANT SANITATION

Microbiological tests can prove of great value in determining the sanitary condition of the plant and equipment, and for ensuring that the risk of contamination from this source is reduced to a minimum. We have already mentioned earlier in this paper that yeasts, in particular, accumulate rapidly on the cheese cloths, and these should be thoroughly washed at the end of each days' pressing. Forgaes *(3)* has shown the superiority of stainless steel and enamel equipment over wooden vats, and our own observations have confirmed that the latter are not only a serious source of contamination but are impossible to maintain in a sanitary condition. After use, stainless steel and enamel vats should be thoroughly hosed with a powerful jet of cold water followed by scrubbing with hot water and treatment with live steam. Periodic examination of the interior of such vessels after this treatment, by means of swabs and disintegration of the swabs in sterile water and plating out, rarely shows a count exceeding four yeasts or molds per square inch of surface. In addition to the daily cleaning it is also desirable to treat the vats once weekly with dilute soda, followed by scrubbing and with hot and cold water rinsing.

Filters are difficult to keep clean, but if they are drained and thoroughly hosed daily, and the plates immersed in hot water for at least 15 minutes we have found that it is possible to attain a condition of sanitation comparable to that found in the stainless steel vats.

Juice pipes should, wherever possible, be regularly sterilized with live steam or at least flushed for 15 minutes with hot water.

The filling tubes on bottling machines are notorious breeding grounds for all types of apple juice spoilage organisms and these, after thorough flushing with cold water, should be removed and immersed overnight in hot water. It is occasionally advisable to carry out microbiological examinations of bottles after washing using either the rinse method or the direct incubation method as described by Baumgartner *(1)*. This test is particularly useful on old bottles in which yeast and mold colonies have dried out with the passage of time and sometimes become difficult to remove unless the bottle washer is working at peak efficiency.

We have found it desirable to aim at achieving the standards prescribed by the American Public Health Association for sanitation of dairy equipment. This Association has stated that counts of less than 100 organisms per 4 square inches indicate satisfactory cleansing. Our own records have shown that with stainless steel or enamelled lined equipment it is practical to attain similar standards of plant sanitation in apple juice factories.

SUMMARY

The microbial population of apple juice should diminish continually throughout processing if the latter is rigidly controlled and precautions to arrest development of microorganisms are adequate. Routine microbiological counts at each processing stage are of value in checking the efficiency of the plant and for ensuring that the latter is maintained in a sanitary condition.

Acknowledgment

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PROTEIN DENATURATION IN FROZEN AND STORED FISH[a]

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Study of the per capita consumption of frozen fish in different areas of Canada shows that it is not a popular food. Particularly in the inland centers of Ontario and Quebec where fresh and frozen fish compete side by side, the preference for fresh fish is well shown. This is true in spite of the difficulty in shipping fresh fillets this distance in reasonably fresh condition. For example, in Toronto in 1947 the per capita consumption of fresh Atlantic cod fillets was 1.4 lbs., whereas frozen cod fillets totalled only 0.34 lb. Similarly, in the city of Montreal, the corresponding consumption was 2.2 and 0.37 lbs., respectively. In the province of Nova Scotia, close to the supply of both products, the figures were 0.74 lb. and 0.50 lb., respectively *(10)*. It is evident that there exists a very decided preference for the fresh over the frozen product.

Several laboratories including our own have been working on this problem. There is ample evidence that the act of freezing itself does not account for the lowering in quality, except in a few species of minor importance *(12)*. In fact, the quality may often be improved immediately after freezing *(11)*.

Young *(11)*, by means of taste panels on frozen and fresh halibut, showed that freshly frozen samples were somewhat firmer and drier than the unfrozen ones and were preferred by many people. While the appearance of rapidly frozen samples stored at 14°F.(−10°C.) was superior to that of slow frozen samples stored at −13°F.(−25°C.), the taste panel tests were very definitely in disagreement. With Pacific salmon also *(12)* it was found that after an initial period, the temperature of storage became more important than the rate of freezing.

Notevarp and Heen *(4)* have studied the effects of freezing rate, storage temperature, and freshness of raw material on the quality of frozen cod and herring. They showed that changes in freezing rates up to where 8 hours are required for freezing have almost no effect on the product. Of course, very slow freezing is detrimental, but storage temperature rapidly becomes more important than the rate of freezing. The state of preservation of the fish previous to freezing greatly affected the quality in frozen storage. At 16°F.(−9°C.), fresh cod remained acceptable as judged by a taste panel for 2.5 to 3.0 months; frozen after 3 days of prestorage in ice, it kept for about 2 months; and after 8 days in ice it kept for only about 1 month. At −4°F.(−20°C.), fresh cod kept well for 8 to 9 months; after 3 days in ice, about 5 months; and after prestorage for 8 days in ice, about 3 to 4 months.

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Reay *(5)* summarizes experiments carried out at the Torry Fisheries Research Station in Britain on freezing of white fish, and concludes that freshness of fish and temperature of storage are most important, the rate of freezing being a minor factor.

In our laboratory, we have carried out several experiments using taste panels to measure the deterioration in quality of Atlantic cod, haddock, and Atlantic halibut in frozen storage.

In the first series of tests, it was shown that haddock had a keeping quality definitely superior to cod, and it was found that the temperature of storage soon became much more important than the freezing rate. While large ice crystals developed on slow freezing and gave the fish a poor appearance, this had little effect on the taste panel rating. Our conclusions therefore agree with Young *(11)*, Notevarp and Heen *(4)*, and Reay *(5)* that the rate of freezing is not as important as it was considered formerly. Prebrining the fish to prevent loss of drip on thawing overcomes most of the ill effects of moderately slow freezing, although some sacrifice of appearance is made.

In the second series, strictly fresh cod were used. The samples were rapidly frozen in 1-lb. flat cans to avoid the effects of surface desiccation. Two storage temperatures of 10°F.(−12°C.) and −10°F.(−23°C.) were used. The fish samples were baked at 500°F.(260°C.) for 16 minutes and served without condiments along with a fresh control. Three panels of about 8 judges were used for each sampling. The samples were graded on the basis of texture, odor, and taste, and were given ratings of 0 to 5.

At 10°F.(−12°C.) the samples became inferior to the control in three weeks of storage and were unacceptable after about five months (Figure 1). The samples stored at −10°F.(−23°C.) were superior to the control for about 6 weeks, being slightly more tender, and equalled the control up to about 5 months. They became unpalatable at 10 to 11 months.

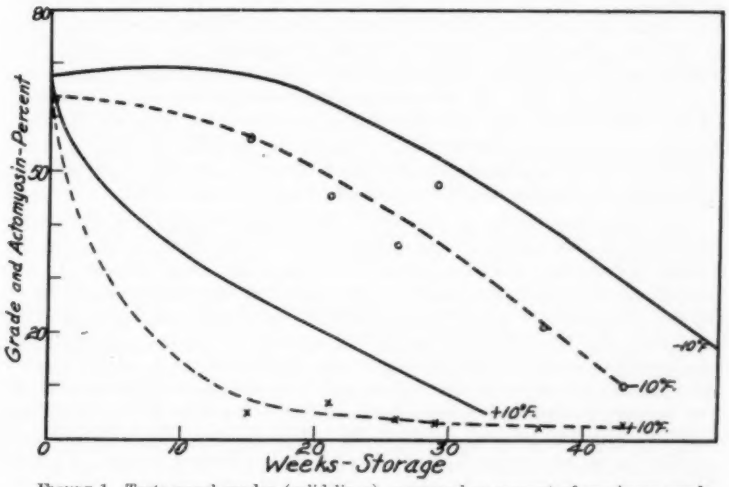

FIGURE 1. Taste panel grades (solid lines), expressed as percent of maximum grade, and soluble actomyosin (dotted lines), expressed as percent total protein, in frozen cod fillets stored at 10°F.(−12°C.) and at −10°F.(−23°C.).

The important changes occurring were the development of tastelessness and loss of tenderness, finally proceeding to development of toughness and of off-flavors and odors.

Halibut was examined after storage at temperatures of 10°F.(−12°C.), 0°F.(−18°C.), and −10°F.(−23°C.). Here again, the texture change from tender to tough and development of objectionable off-flavors were the major factors in the taste panel assessments. Atlantic halibut in sealed 1-lb. cans kept satisfactorily for 18 months at −10°F. (−23°C.) and at 0°F.(−18°C.) as shown in Figure 2. At 10°F.(−12°C.) the samples were rejected after about 8 months. There was insufficient difference between storage temperature of 0°F.(−18°C.) and −10°F.(−23°C.) to justify the use of the lower temperature.

Two things are evident from this work: 1, that off-flavors suggestive of fat spoilage develop on frozen storage even in the non-fatty fish such as cod and haddock; and 2, the lowering of grade parallels the loss of tenderness and the development of toughness.

In the cod stored for 9 months, it was found that the fat contained about 72% free fatty acids calculated as oleic acid. Similarly, in the halibut there was a development of free fatty acids and an increase in peroxide values on storage. Thus, fat spoilage definitely cannot be ruled out in the frozen fish in the low fat species such as cod and haddock.

The second finding, the development of toughness, is related to the denaturation of the proteins. This has been studied extensively.

Reay and Kuchel *(6)*, applying to fish the procedure used by Bate-Smith *(7)* in his study of the proteins of rabbit muscle, found a decrease in the solubility of the proteins extracted by salt solutions after frozen storage. Dyer, French, and Snow *(3)* investigated this further and showed that it was the actomyosin fraction which was denatured, the non-actomyosin fraction remaining unchanged except after very long storage. The protein fractions present were also determined using a method by which 90 to 95% of the total protein was dispersed in 5% sodium chloride solution. Actomyosin was found to comprise 75 to 80% of the protein. Extraction of the soluble protein, followed by an estimation of the actomyosin of the solution then gives us a measure of the degree of protein denaturation.

The results obtained in the above taste panel experiments are shown in Figure 1 (dotted lines) and Figure 2. It is seen that in all cases the soluble actomyosin decreases faster than the taste panel grades. In the case of the cod fillets, the soluble actomyosin has decreased almost to zero by the time the taste panel had rejected the samples as unacceptable. It is thus seen that the actomyosin solubility appears to parallel and anticipate the taste panel rating and provides a quantitative measure of the quality of a particular sample of fish. Of course, it is only one of the variables in the complex function of consumer acceptability, but it is an important one and one which in general parallels the other changes. Work is now proceeding in testing this method of assessing and predicting quality of frozen fillets.

Considerably more work has been carried out on the denaturation of actomyosin systems both in situ and in isolated form. It has been already

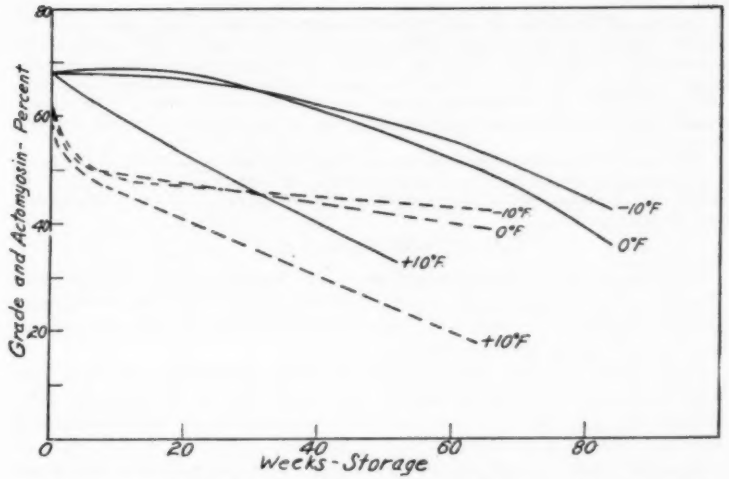

FIGURE 2. Taste panel grades (solid lines), expressed as percent of maximum grade, and soluble actomyosin (dotted lines), expressed as percent total protein, in frozen Atlantic halibut stored at 10°F.(−12°C.), 0°F.(−18°C.), and −10°F.(−23°C.).

established, for instance, that surface dehydration almost completely denatures the proteins on the surface.

When actomyosin is extracted in salt solution, it may be precipitated by diluting with cold water to a concentration of about 0.5% sodium chloride. On standing, the actomyosin may be centrifuged off as a gel which still contains about 99% water.

When gels of actomyosin are frozen, they are almost completely denatured. But not so with sols. Here little or no denaturation occurs, depending on the salt present and other conditions. In the muscle cell, the actomyosin exists mostly in the form of a sol and thus the denaturation of the actomyosin in situ parallels the sol denaturation rather than that of the gel.

It was found *(8)* when sols of actomyosin were frozen in the presence of various salts, that denaturation did not occur as long as the temperature was kept above the eutectic point. When this was passed, however, rapid denaturation occurred, presumably because the sol was now converted to the gel form.

Since we do not know the concentration of each of the various salts in fish muscle, or their eutectic points, we cannot say whether there is a temperature below which denaturation would occur. Furthermore, different species may vary in this respect.

Another line of investigation which has been pursued is the effect of salt on actomyosin. Duerr and Dyer *(2)* have shown that when cod fillets are salted in saturated brine there is a definite concentration of salt (about 8 to 10%) in the fillet at which denaturation occurs. As the salt concentration reaches this level, the actomyosin is suddenly denatured, the water held by the protein micelles is released, and diffuses into the more saturated brine outside. Thus, a rapid loss of water occurs at this point.

This was carried further with isolated actomyosin sols and it was shown that the salt concentration necessary for denaturation decreased with increasing concentration of protein such that a supersaturated sol as present in fish muscle would be denatured at about 10% salt. Determination of moisture contents of the denatured gels showed that the moisture content decreased rapidly with increasing salt content and it would appear that a competition for water by the myosin and the salt solution exists.

As the salt penetrates the gel, the salt concentration rises and in the region of 3 to 8% sodium chloride concentration the myosin swells strongly. But when the salt concentration reaches about 10% the swelling is strongly reduced, the actomyosin releases sufficient of its water to disrupt the native configuration of its highly organized micellar structure and denaturation occurs. This same process is related to the denaturation occurring on freezing which may partially be considered as a removal of water as ice crystals.

The moisture contents of gels denatured by freezing and by salt were determined. Freeze-denatured gel was found to contain 95.0% water whereas the native gel contained 98.7%. Salt denatured gels varied from 88 to 94% depending on the salt concentration used to denature the gel.

DISCUSSION

Of course, one of the uncertainties in this work is a lack of knowledge of what protein fractions are actually present. Recent work on actomyosin by Szent-Györgyi *(9)* and Dubuisson *(1)* emphasizes the involved chemistry of the muscle protein complex.

For instance, lipids, fatty acids, nucleotide compounds, or even carbohydrates may protect or modify the properties of actomyosin. The understanding of these questions is just beginning to be unravelled, and nobody can say where the future lies. It seems quite within the realm of probability that we may be able to inhibit, at least partially, the denaturation in frozen products.

Other problems which we should consider in dealing with frozen fish products are, of course, the engineering aspects of the efficient design of freezing apparatus and of temperature maintenance during storage. Advances are being constantly made and as an example one might mention the development of refrigerated railway cars and trucks capable of maintaining sustained low temperatures.

SUMMARY

The above discussion suggests certain points to be kept in mind in the freezing and storage of fish. Fresh material should be used and reasonably fast freezing should be applied. Storage should be at 0°F.(—18°C.) or below. Desiccation should be prevented and the storage period should be limited according to the specific requirement of the species. Low refrigeration temperatures should be maintained throughout the merchandizing chain.

Attention to these factors will reduce the deterioration in quality of frozen fish products occurring on storage. There will be less protein denaturation, and therefore less toughening of the texture and off-flavor development will be minimized as far as our present knowledge will permit. Even under the best storage and handling conditions, however, deterioration does occur. Little can be done to advance our knowledge until we know more of the physical chemistry of the proteins present. This is the line of endeavor on which we are working at present.

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A new and expanded edition of the pamphlet "Information and suggestions for authors" has been published for those writing technical contributions for *Food Technology* and *Food Research*.

The pamphlet discusses the respective scopes of the two journals and the type of articles suitable for them. A detailed outline is presented of the customary organization of technical papers followed by statements concerning the preferred styles and methods of preparing manuscripts. A discussion of various details like units of measurements and their abbreviations, use of footnotes and literature citations, preparation of tables and figures, etc. follows.

In the following chapter the customary methods of correcting printer's proofs are described. Here a list is given indicating the more common corrections. Instructions for the ordering of reprints follow.

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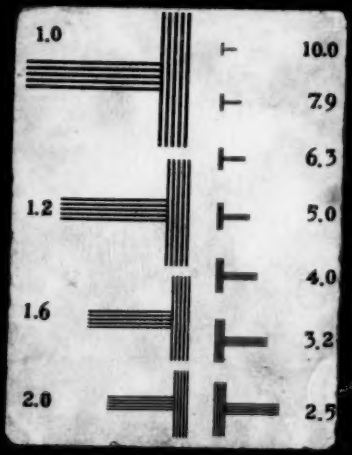

100 MILLIMETERS

INSTRUCTIONS Resolution is expressed in terms of the lines per millimeter recorded by a particular film under specified conditions. Numerals in chart indicate the number of lines per millimeter in adjacent "T-shaped" groupings.

In microfilming, it is necessary to determine the reduction ratio and multiply the number of lines in the chart by this value to find the number of lines recorded by the film. As an aid in determining the reduction ratio, the line above is 100 millimeters in length. Measuring this line in the film image and dividing the length into 100 gives the reduction ratio. Example: the line is 20 mm. long in the film image, and 100/20 = 5.

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